THE EFFECTS OF GLUCOSE, FRUCTOSE, AND GALACTOSE ON THE RESPIRATORY EXCHANGE OF THE GOAT [1]

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In pursuance of the Nutrition Laboratory's general study of the biological variations in the effects of hexoses on the respiratory exchanges of animals of different species, the investigation here reported was made on the goat as a representative of ruminants. As the diet of adult ruminants is mainly of plant origin, it is conceivable that the rate of utilization of sugars by the ruminant might be different from that by other species of animal such as man, the rat, or some of the Carnivora. The respiratory exchange of the goat under various conditions has been studied extensively by Magee ('24a, '24b, '24c, '24d) and Magee and Orr ('24), but in no instance was an investigation made with pure sugars.

EXPERIMENTAL

The goats used were four adult males and five adult females, weighing from 33 to 45 kg. Prior to the experiments they were brought from the barn and kept in the laboratory for 40 hours, without food. The goat selected for the day's experiment was placed in the respiration chamber in the morning, and base-line determinations of the respiratory exchange were made in from three to six successive ½-hour periods. The goat was then given the dose for the day, which was 25 gm. of glucose, fructose, or galactose dissolved in 125 ml. of water at 37°–38°C. In the first three experiments the goats were held quietly on their haunches and were fed the solution slowly, a few spoonfuls at a time. This was done in the belief that the liquid would follow the esophageal groove and thus go directly into the true stomach. In the subsequent experiments the solution was given by stomach tube, which was inserted as far as it would go. The goats were held in a sitting position and tilted so that the liquid presumably should have followed a groove

[1] A preliminary communication regarding this investigation was presented at the fifty-second annual meeting of the American Physiological Society. Am. J. Physiol., vol. 129, pp. P329–P330 (1940).

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that would by-pass the rumen. As the reticulum and omasum are relatively small, it is probable that the solution was not much delayed in reaching the fourth stomach or abomasum, provided that the attempted by-passing of the rumen was successful. The results of the respiratory exchange measurements appear to indicate that this by-passing was successfully accomplished, although the solution may have been delayed longer in reaching the fourth stomach than it would have been if the tube could have been inserted directly into the abomasum. After the solution of sugar was given, the funnel and the stomach tube were rinsed with an additional 125 ml. of water. Usually from 4 to 7 minutes were required to introduce the solution, and measurements of the respiratory exchange began from 21 to 31 minutes after ingestion. There were then eight successive ½-hour periods of measurement. Experiments with 250 ml. of water at 37°–38°C. were made as a control series, but in these there were no base-line determinations. The amount of sugar given to the goats was calculated from the amount of sugar commonly given in experiments with man (50 gm.) and the relation of the basal metabolism of the goat to that of man. The environmental temperature at which the goats were studied was approximately 20°C., with variations of ± 3°C.

The measurements of the respiratory exchange were made with an open-circuit respiration apparatus (Ritzman, Washburn, and Benedict, '36), and proportionate samples of the ventilating air current were obtained by the bag method of Fox (Carpenter and Fox, '31a). Analyses of these samples of air were made by an accurate gas-analysis apparatus (Carpenter, '33), which was standardized each day by analyses of samples of outdoor air. In a number of experiments determinations of methane were also made by gas analysis (Carpenter, '33).

In the comparisons made between goats and man in the following pages, the statements regarding man are based on articles by Carpenter and Fox ('30a, '30b, '31b, '31c), Carpenter and Lee ('32, '37a, '37b, '37c, '38), and Root and Carpenter ('42).

RESULTS

Respiratory quotient (R. Q.)

The respiratory quotients in sixteen experiments are given in table 1. The average base-line R. Q.'s in the individual experiments varied from 0.71 to 0.79. The average base-line for two experiments in the series with glucose was 0.75, for the series with fructose 0.77, and with galactose 0.74. The protocols show that the base-line quotients in the successive ½-hour periods were uniform in five experiments, but in the

other three in which base-line values were obtained the ranges were wider than would be expected for the post-absorptive condition. It is to be questioned whether the goat is in the post-absorptive condition 40 hours after withdrawal of food, although all the average base-line R. Q.'s for the individual days were low enough to indicate complete absence of effect of the previous meal.

The effect on the R. Q. of ingestion of 250 ml. of water was slow in reaching its peak, as the maximum R. Q. did not occur in three experiments until the third to the sixth ½-hour period after ingestion. In the other two experiments the R. Q. was steady. The averages of the R. Q. for the last three experiments with water were 0.73 in the first

TABLE 1

Respiratory quotients before and after ingestion of water or sugar by goats. (Without food 40 hours at start of expt.)

DOSE[1] AND DATE	BASE-LINE (AVG.)	HALF-HOUR PERIODS AFTER INGESTION							
		1[2]	2	3	4	5	6	7	8
1938									
Water[3]									
June 29	...	0.71	0.70	0.70	0.71	0.71	0.71	...	...
June 30	...	0.69	0.68	0.69	0.71	0.72	0.70	...	...
Oct. 25	...	0.72	0.71	0.75	0.77	0.77	0.78	0.74	0.73
Oct. 29	...	0.72	0.73	0.74	0.75	0.77	0.76	0.76	0.74
Nov. 4	...	0.75	0.75	0.77	0.77	0.77	0.75	0.76	0.76
Glucose[4]									
Feb. 25	...	0.75	0.80	0.86	0.86	0.85	0.83	0.83	0.82
June 29	...	0.68	0.69	0.76	0.75	0.74	0.75	0.75	0.72
June 30	...	0.72	0.71	0.74	0.76	0.76	0.75	0.76	0.73
Oct. 27	0.75	0.74	0.80	0.84	0.83	0.83	0.86	0.82	0.83
Nov. 2	0.74	0.77	0.78	0.79	0.79	0.79	0.81	0.79	0.79
Fructose[4]									
Oct. 24	0.79	0.80	0.83	0.86	0.85	0.87	0.86	0.84	0.84
Oct. 28	0.74	0.81	0.81	0.85	0.82	0.82	0.81	0.79	0.80
Galactose[4]									
Oct. 26[5]	0.73	0.77	0.72	0.75	0.70	0.75	0.77	0.77	0.75
Oct. 31	0.75	0.76	0.77	0.80	0.80	0.83	0.82	0.83	0.82
Nov. 1	0.71	0.73	0.72	0.76	0.75	0.76	0.76	0.77	0.76
Nov. 3	0.76	0.76	0.79	0.78	0.82	0.81	0.81	0.80	0.78

[1] In the experiments on Feb. 25, June 29, and June 30 the goat was given the solution to drink a few spoonfuls at a time; in all other experiments the solution was introduced by stomach tube.

[2] The first half-hour period began 21 to 31 min. after ingestion.

[3] 250 ml. of water at 37°–38°C.

[4] 25 gm. of sugar in 125 ml. of water plus 125 ml. of water for rinse, at 37°–38°C.

[5] Solution of galactose may not have been introduced into the true stomach.

two periods and 0.76, 0.77, and 0.76 in the fourth, fifth, and sixth periods. Analyses for periods 5, 7, and 8, respectively, of the last three experiments with water showed a complete absence of methane in the expiratory gases and in period 8 on October 25th only 0.012% of methane.

The effect on the R. Q. of the ingestion of 25 gm. of glucose was evident in three experiments by the third period and reached its maximum in the third and fourth periods in three experiments and in the sixth period in two experiments. In the two experiments in which base-line values were obtained, the maximum rises above the base-line were 0.11 and 0.07. The average quotients for the successive periods after ingestion of glucose were 0.73, 0.76, 0.80, 0.80, 0.79, 0.80, 0.79, and 0.78. The rises in the quotients of these goats in this series of experiments were nearly as large as those noted with man after the administration of 50, 62, or 100 gm. of glucose. It is assumed that these are true metabolic quotients, as an attempt was made to introduce the solution of glucose into the true stomach and thus avoid fermentation. Unfortunately the methane content of the chamber air was determined in only three periods in the glucose experiments (period 8 after glucose on October 27th and periods 4 and 7 on November 2nd). In these instances it was found to be 0.007, 0.003, and 0.024%, respectively. It is questionable whether the maximum amount is significant.

There are some indications that the metabolism of carbohydrates is different in ruminants from that in other mammals. Krzywanek and Brüggemann ('33) report that the administration of glucose into the true stomach of the sheep had no appreciable effect on the level of blood sugar. This absence of effect they ascribe to fermentation of the sugar in the stomach of the ruminant. Cutler ('34) found that the normal resting blood sugar of twenty-six goats ranged in the majority of cases from 40 to 50 mg. per 100 ml. and that the oral administration of 1.5 gm. of glucose per kilogram of body weight caused the blood sugar to rise from a base-line of 42 mg. to only 59 mg. She offered as an explanation that the goat is a ruminant and, therefore, possibly the rate of absorption is relatively slow. Magee ('32) found that the blood sugar of goats rose from a base-line of 80 mg. to 110 mg. per 100 ml. after 50 gm. of sucrose and 150 gm. of starch, when the animal had fasted 48 hours. After longer fasting, the rise was not so great.

The effect on the R. Q. of the ingestion of 25 gm. of fructose was evident promptly, as there was a definite rise above the base-line in the first and second periods after the dose. The maximum occurred in the third and fifth periods. The average quotients after fructose

were, by periods, 0.81, 0.82, 0.86, 0.84, 0.85, 0.84, 0.82, and 0.82. Significant amounts of methane were detectable in only two of the periods after fructose in which these analyses were made (October 24th, periods 3 and 8, 0.007 and 0.041%; October 28th, periods 4 and 8, 0.006 and 0.025%). The maximum rise was lower and occurred later with the goat than with man after administration of 50 or 100 gm. of fructose. The effect of fructose on the R. Q. of the goat resembles more nearly that found with cats (unpublished experiments) than that noted with man and with monkeys (Carpenter and Hartman, '44).

The effect of the ingestion of 25 gm. of galactose was evident in all four experiments by the fourth period. The results in the experiment on October 26th were irregular. It is possible that the solution might not have been introduced into the true stomach. However, the two analyses made for methane in this experiment indicate no fermentation. The last four periods in this experiment show a distinct rise in the R. Q. The maximum rises in the other three experiments were 0.08, 0.06, and 0.06 in the fifth, seventh, and fourth periods, respectively, after administration of the sugar. The average quotients after galactose were, by periods, 0.76, 0.75, 0.77, 0.77, 0.79, 0.79, 0.79, and 0.78. These resemble closely those after the ingestion of 25 gm. of glucose. Analyses for methane were made in a total of nine periods. In the several experiments, in the third period after ingestion of galactose, the methane values were 0.0, 0.015, 0.013, and 0.015%, respectively, and in the sixth or the seventh period they were 0.0, 0.031, 0.012, and 0.035%, respectively. In the eighth period on November 1st, one analysis showed 0.008%. In other words, in three experiments a measurable amount of methane was found in the third and the sixth or the seventh period after ingestion of galactose, but in the experiment in which it was doubted whether the solution was placed in the true stomach no methane was found. The finding of methane in several periods indicates that the metabolism of galactose in these goats was accompanied by fermentation. The effects of such amounts of methane on the R. Q. are negligible. The changes in the quotients of goats after galactose ingestion differ from those with man after 50 gm. of galactose, as the rise with man is much greater and the peak occurs earlier than with goats.

Heat production and metabolism of carbohydrates

The values for the heat production in the basal periods, calculated to the basis of 4 hours from the measurements of the respiratory exchange, and for the heat production during the 4 hours of measure-

ment after the ingestion of water and of the sugars are given in table 2. The values are not corrected for activity, as the purpose of the calculation of the heat production was to estimate the metabolism of the carbohydrates. Because of the variability in activity, no conclusions can be drawn as to the effects of ingestion of water or of the sugars on the output of energy. The metabolism of carbohydrates was calculated in the usual empirical manner from the measurements of the respiratory exchange, after correction for the metabolism of protein on the assumption that 15% of the oxygen absorption was ascribable to the oxidation of protein. The values are given in table 2.

TABLE 2

Heat production and metabolism of carbohydrates before and after ingestion of water or sugar by goats.
(Values per 4 hours)

DOSE[1] AND DATE	GOAT NO.	SEX	BODY WEIGHT	HEAT PRODUCTION		CARBOHYDRATE METABOLIZED	
				Baseline	After dose	Baseline	After dose
1938			*kg.*	*cal.*	*cal.*	*gm.*	*gm.*
Water							
June 29	8	M	33.3	...	198	...	0.0
June 30	9	M	33.3	...	201	...	0.0
Oct. 25	2	M	37.0	...	177	...	3.6
Oct. 29	6	F	41.5	...	189	...	3.8
Nov. 4	5	F	45.1	...	207	...	6.5
Glucose							
Feb. 25	7	F	42.9	...	177	...	14.8[1]
June 29	8	M	33.3	...	204	...	1.7[1]
June 30	9	M	33.3	...	196	...	3.1[1]
Oct. 27	4	F	39.2	179	173	3.5	13.2
Nov. 2	3	F	35.8	144	149	2.3	8.3
Fructose							
Oct. 24	2	M	39.2	197	201	10.6	19.8
Oct. 28	5	F	43.8	217	227	4.0	16.9
Galactose							
Oct. 26	3	F	35.4	186[2]	165[2]	3.0	4.2[2]
Oct. 31	1	M	39.5	167	168	3.6	11.4
Nov. 1	2	M	36.7	189[2]	168	6.0	3.8
Nov. 3	4	F	40.6	164	166	5.0	9.5

[1] In the experiments on Feb. 25, June 29, and June 30 the goat was given the solution to drink a few spoonfuls at a time; in all other experiments the solution was introduced by stomach tube.

[2] Goat active.

[3] Solution of galactose may not have been introduced into the true stomach.

The amounts of carbohydrate utilized in 4 hours in the base-line periods varied from 0 to 10.6 gm. The majority were under 4 gm. As no base-line values were determined in the experiments with water, no conclusion can be drawn regarding the effect of ingestion of water on the metabolism of carbohydrates. However, from the changes in the R. Q. in the experiments with water and from the general level of the base-line values for carbohydrate metabolized in the other series of experiments, there is evidence in three of the experiments that the metabolism of carbohydrates was slightly increased after the ingestion of 250 ml. of water. In the experiments with the sugars, the metabolism of carbohydrates was highest after the ingestion of fructose, both from the standpoint of absolute values and from the standpoint of increases above the base-line values. Even when the base-line value was high (10.6 gm. with a base-line R. Q. of 0.79), there was an increase of 9.2 gm. The carbohydrate metabolized after ingestion of glucose was the next in order of increase above the base-line, if the experiments in which the sugar was not given by stomach tube are disregarded. After ingestion of galactose the increases were the least, except in one experiment where the increase was 7.8 gm. With man the greatest increase is after fructose ingestion, whereas the increases after administration of the other two sugars are less and about the same. The rate of metabolism of carbohydrate after fructose ingestion is so high with goats that 25 gm. of this sugar might not suffice for much longer than 8 hours, whereas 25 gm. of galactose might supply the necessary carbohydrate for from 12 to 24 hours.

SUMMARY

The respiratory exchanges of four male and five female adult goats were determined 40 hours after withdrawal from food (1) under basal conditions and (2) in eight successive ½-hour periods after the administration by stomach tube of 250 ml. of water at 37°–38°C., or of 25 gm. of glucose, fructose, or galactose dissolved in 125 ml. of water and an additional 125 ml. of water for rinsing.

Water produced a slight but somewhat delayed increase in the R. Q. Fructose caused the greatest increase in the R. Q. and the greatest increase in the metabolism of carbohydrates. Glucose was next in these effects, and galactose had the least effects. Qualitatively these results resemble much those found with man with these sugars. There was evidence of a slight amount of fermentation after the ingestion of galactose and of fructose by the goats.

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THE ROLE OF DIETARY FAT AND LINOLEIC ACID IN THE LACTATION OF THE RAT

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In a long series of experiments summarized by Maynard and coworkers in 1941 it has been shown that dietary fat stimulates milk secretion in dairy cows. A somewhat greater quantity of milk was consistently produced on the higher fat diet. Maynard and Rasmussen ('42) used the growth rate of standardized litters to demonstrate that high-fat diets support better lactation performance in rats than low-fat diets. Corn oil and crude coconut oil seemed to have a similar effect despite their widely different fatty acid makeup.

In order to investigate possible explanations of the effect of fat on lactation performance experiments were set up to measure the growth of litters whose mothers were fed a diet free from fat as compared with varying amounts of corn oil, and hydrogenated fat. The composition of the diets is shown in table 1.

METHODS

Mature female rats which had previously littered were mated and caged separately before parturition. After 12 but before 24 hours following parturition, the feed was removed and the litters standardized to six. Females were selected in pairs, triplets or quadruplets that were similar in body weight and in weight of litter for comparison of the diets. The females were fed equicaloric amounts of food usually corresponding to that consumed by the female on the fat-free diet. The young and mothers were weighed daily during a 17-day period to follow growth increases and to note individual characteristics of the litter. On the morning ending the 17-day period the young were etherized, the viscera removed and the carcasses frozen until chemical analyses were made. The results obtained with four females on each treatment are shown in table 2.

It is seen that when the diet contained 5.5, 11.3, or 19.2% of corn oil the growth of the litters was definitely superior to that on the low

fat diet. This suggested that an essential nutrient element was being supplied to satisfy metabolic needs at all levels of corn oil fed.

Comparisons were then made of the low-fat, corn oil and hydrogenated corn oil diets using trios of rats. The results (table 2) show the superiority of the corn oil over the low-fat diet. On the diet containing hydrogenated corn oil the young made less gain in weight than on the low-fat diet and the mothers lost more weight. These results cannot be given much reliance because balance studies revealed that as an average only 54.3% of the hydrogenated corn oil was absorbed, and, therefore, the calories available for maintenance and lactation were appreciably less than for the mothers fed the low-fat or corn oil diets.

Hydrogenated coconut oil was next selected for comparison because of its relatively low melting point and high digestibility (Hoagland and Snider, '43). In this comparison (table 2) less growth was obtained on the diet containing hydrogenated coconut oil than on the low-fat diet.

Carcass analyses of the young from these three studies showed that most of the extra gain in weight of the young on the corn oil diet was due to the deposition of extra body fat. There was only a slight increase in total protein deposited.

These data confirmed the earlier study from this laboratory in showing that corn oil in the diet of rats stimulates lactation performance. It appeared that something in corn oil, lost by hydrogenation, was responsible for the faster growth of the young rats. The possibility of a deficiency of the essential fatty acids presented itself.

Linoleic acid not the first limiting factor

To test the possibility that a deficiency of linoleic acid was retarding the growth of the young, two female rats with litters standardized to six were at parturition placed on a fat-free diet (described later). Each litter was divided into two groups of three. To one group of the young nursed by each mother was fed 1 drop of ethyl linolate per day. To the other group was given 1 drop of fully hydrogenated coconut oil per day. The feeding period was extended to 17 days. In neither case could any significant advantage be assessed to the group receiving the ethyl linolate.

On the basis of this preliminary test it was felt that any advantage due to ethyl linolate would result from a stimulus to the lactation process, and that poor growth of the young on the low-fat diet was not due to a deficiency of the essential fatty acids in the milk.

To test the lactation response due to linoleic acid and to obtain more data on the value of hydrogenated coconut oil four different nutrient comparisons were studied. They were, an ether extracted purified diet, the same supplemented with 125 mg. of ethyl linolate for each rat daily, a purified diet containing 10% hydrogenated coconut oil, and a purified diet containing 10% corn oil. The same low-fat diet shown in table 1 was used except that the corn starch was replaced with

TABLE 1

Composition of diets.

INGREDIENT	LOW FAT	CORN OIL	CORN OIL	CORN OIL	HYDROGENATED CORN OIL [1]	HYDROGENATED COCONUT OIL [2]
	parts	*parts*	*parts*	*parts*	*parts*	*parts*
Casein	20	20	20	20	20	20
Yeast (extracted)	5	5	5	5	5	5
Corn starch	40	25	30	35	25	36.3
Sucrose	30	11.3	17	23.4	11.3	. . .
Bone meal (extracted)	2	2	2	2	2	2
Salts (Hawk and Osler)	3	3	3	3	3	3
Choline	0.1	0.1	0.1	0.1	0.1	0.1
Cystine	0.2	0.2	0.2	0.2	0.2	0.2
Fat	. .	15	10	5	15	15
Total	100.3	81.6	87.3	93.7	81.6	81.5
Protein %	16.9	23.3	21.6	20.4	21.8	24.7
Fat %	0.2	19.2	11.3	5.5	17.3	18.5
Ash %	5.2	5.4	5.8	4.6	5.7	5.5

[1] The corn oil was hydrogenated in our laboratory to an I.V. of 30.

[2] The hydrogenated coconut oil (I.V. 0.6) was kindly furnished by Lever Brothers, New York, N. Y.

Each female received daily 40 I.U. of vitamin A, 4 I.U. of vitamin D, 0.2 mg. of alpha-tocopherol and 15 drops of rice bran concentrate, "Vitab". The females were irradiated for 30 minutes three times each week.

sucrose. In the high-fat diet 10 parts of the oil replaced an equicaloric amount of sucrose. The casein, yeast and bonemeal were extracted with ethyl ether continuously for 48 hours. The corn oil used in diet 3 was a purified grade with an iodine value of 126.3. The hydrogenated coconut oil used had an iodine value of 0.6. The Osborne and Mendel salt mixture was used. Generally a fat-free base was mixed. The appropriate amount of sucrose or fat to give the three diets was then added to it. The nutritive ratios of these diets were so adjusted that they would be the same when equal caloric intakes were fed. One gram

of the low-fat diet was fed to supply the same number of calories as 0.899 gm. of the high-fat diets. Analyses showed that the ether-extracted diet contained 18.2% of protein, 0.2% fat and 76.5% carbohydrates, the corn oil diet 22.0% of protein, 10.2% of fat and 61.2% carbohydrates.

The mother rats received supplements of the fat soluble vitamins dissolved in hydrogenated coconut oil. One drop of the supplements was given three times each week to supply 123 μg. B-carotene, 969 μg. 2 methyl 1–4 napthoquinone, 21 mg. tocopherol, and 708 U.S.P. units of vitamin D weekly. Vitamin K was included in this supplement in view of the report of Harris and Mosher ('40) that rats may develop

TABLE 2

The average growth and composition of litters of rats on an ether extracted diet and with added corn oil, hydrogenated corn oil or coconut oil.

DIET	PERCENT FAT	CALORIC INTAKE	PROTEIN INTAKE	CHANGE IN WEIGHT OF MOTHERS	GAIN OF LITTER	CARCASS CONTENT OF	
						Protein	Fat [1]
			gm.	*gm.*	*gm.*	*gm.*	*gm.*
Ether extracted	0.2	1707	83.0	— 6	121.9	17.9	16.0
Corn oil	5.5	1752	93.6	+ 3	152.1	21.3	24.8
Corn oil	11.3	1762	94.0	— 8	161.2	20.7	29.0
Corn oil	19.2	1692	89.3	— 17	150.1	20.7	24.8
Ether extracted	0.2	1854	90.0	+ 3	131.7	20.1	17.6
Corn oil	19.2	1847	97.4	— 15	164.8	22.2	23.7
Hydrogenated corn oil	17.3	1845	98.0	— 23	114.3	17.7	13.1
Ether extracted	0.2	1546	74.5	— 11	158.0	24.1	22.0
Hydrogenated coconut oil	18.5	1536	86.3	— 8	134.0	20.9	19.0

[1] The dried carcasses were extracted with a mixture of anhydrous ethyl ether 50 parts and absolute ethyl alcohol 50 parts by volume.

a vitamin K deficiency when maintained on a diet containing an abnormal dietary fat constituent (dihydroxy-stearic acid) as its major fatty component. Two drops of a rice bran concentrate [1] were administered daily as a source of B-complex vitamins. Ethyl linolate was fed by dropper in 125 mg. daily dosages.

Mother rats were selected in quadruplets according to body weight, age and weight of litter, one for each dietary. In all, seven animals were fed equal caloric amounts of each diet and the weight change of mother and young recorded as formerly. The average nutrient intakes and weights of the four groups are shown in table 3.

[1] Vitab.

TABLE 3

Nutrient intakes, live weights and weight gains of rats studied.

DIET	TOTAL INTAKE	PROTEIN INTAKE	INITIAL WEIGHT		WEIGHT CHANGE OF MOTHERS	WEIGHT GAIN OF LITTER
			Mother	Litter		
	Cal.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Ether extracted	1253	59.8	247	35.6	— 13	110.6
Hydrogenated coconut oil	1220	63.2	257	35.0	— 21	107.9
Corn oil	1220	63.8	248	33.2	—9	129.1
Ether extracted plus 125 mg. of ethyl linolate	1241	58.4	260	36.4	— 21	112.1
Least difference (odds of 1: 100)	....	...	...	...	24	19.0
Significance of difference			ns	ns	ns	SS

The initial weights of the females and litters were quite similar. As an average, the mothers lost weight while suckling their young but no correlation could be shown between weight loss of the mothers and diets fed (or gain in weight of the litter). This differs from the observation of Maynard and Rasmussen ('42). They found that the mothers fed high fat diets lost more weight, or gained less, during lactation than those fed low-fat diets.

The gain in weight of those litters on the corn oil diet was decidedly greater than those of the other three diets.

It seemed important to measure the digestibility of the coconut oil since a low absorption would decrease the calories available and depress lactation performance. Studies were, therefore, carried out to compare the absorption of corn oil and hydrogenated oil when they constituted 10.2% of the same diets used in the lactation experiments. The average percentage of apparent digestibility determined with seven adult female rats was 92.3 for hydrogenated coconut oil and 94.5 for corn oil. It is clear that the differences in lactation response cannot be explained on the basis of absorption of the energy.

Carcass analyses of the litters reared on the different diets were made to determine the exact nature of the extra gain in body weight of the young suckled by mothers fed corn oil. The frozen carcasses were ground and dried in vacuo under CO_2 at 60°C. for 48 to 72 hours until constant weight was obtained.

For determination of the body fat a saponification method modified after that of Hurst ('33) was used. The ground, dried carcasses (about 50 gm.) were placed in a tall 600 ml. beaker and an equal weight of

KOH, 200 ml. EtOH, and 100 ml. of water were added. They were then heated on a hot plate just below boiling for 1½ to 2 hours, 50 ml. water were then added and the solution boiled for 30 minutes. The solution was filtered through glasswool to remove undissolved particles and washed with hot H_2O, making up to 300 ml. final volume.

The fat was determined using the Rose-Gottlieb wet extraction procedure. Fifteen ml. soap solution were acidified with 15 ml. 40% HCl and 15 ml. 95% EtOH added. Two extractions were made with 50 ml. portions of a mixture (1:1) of ethyl and petroleum ether. The fatty acids were dried under CO_2 at 95°C. for 15 minutes and then weighed.

The iodine values for the extracted body fats and milk fats were determined by the A.O.A.C. method.

The results of the analyses for dry matter, fat, and iodine number are shown in table 4.

TABLE 4

Average cracass composition of the litters.

DIET	FRESH WEIGHT	DRY MATTER	FAT	NON-FAT DRY MATTER	BODY FAT IODINE NO.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*
Ether extracted	130.5	34.0	8.5	25.5	61.7
Coconut oil	128.5	33.4	8.4	25.5	57.3
Corn oil	147.5	41.5	13.4	28.1	80.4
Ether extracted with ethyl linolate	132.8	34.4	8.5	25.9	64.9
Least difference (odds of 1: 100)	....	6.6	3.0	4.7	8.4
Significance of difference	....	SS	SS	ns	SS

The corn oil group contained more total dry matter and total fat than any of the other three groups. However, the non-fat dry matter in the carcasses of all groups was about the same, only a barely significant difference (odds of 1:20) in favor of the corn oil group existed. Practically all of the extra gain in weight of the corn oil group may be accounted for by increase in fat content of the carcasses.

The body fat iodine numbers show some interesting variations. As expected, the group on unsaturated corn oil showed a very significant increase in iodine number. The average iodine number of the group supplemented with ethyl linolate was significantly higher than that of the group on hydrogenated coconut oil. The iodine number of the body fats of the group receiving ethyl linolate was higher than that of the rats receiving only the ether extracted diet but this difference was not significant.

Milk fat and total solids were obtained from the stomach of the young rats killed immediately after they had suckled for a standardized period. The data were so variable that no relation could be shown between the diets fed and the amount of dry matter or fat secreted. The iodine numbers of the milk fats obtained on the respective diets were as follows: ether-extracted diet, 23.1; coconut oil diet, 26.6; corn oil diet, 37.8; ether-extracted diet with ethyl linolate 38.3.

The iodine numbers of the milk fat on the corn oil diet and on the extracted diet supplemented with 125 mg. of ethyl linolate per day were significantly higher than those on the other two diets (the least difference for odds of 1:20 was 11.7). The milk fat on the corn oil diet was always liquid and possessed a yellow-brown coloration while that on the extracted diet was rather clear and semi-solid or solid at room temperature. The litters from the corn oil diets had more oily skins and never developed a temporary scaliness which was sometimes observed in the other litters.

DISCUSSION

The data presented show that suckling rats make more rapid growth when their mothers are fed a purified diet containing corn oil than when fed a fat-free diet. This confirms the findings of Maynard and Rasmussen ('42). That all fats do not have equal values for lactation is shown by the fact that with the diet containing fully hydrogenated coconut oil the growth response was no better than with the fat-free diet. In the earlier study crude coconut oil appeared to be about equal to corn oil. This difference in response suggests that certain unsaturated fatty acids stimulate lactation performance. It appears clear that the more rapid growth of the young which were suckling mothers fed the corn oil diet is due either to the production of more milk or to an alteration in the quality of the milk in such a way that growth of the young is stimulated.

Feeding 125 mg. of ethyl linolate to each lactating female daily did not improve the growth of the young as compared with the ether extracted diet. Thus, this essential fatty acid does not appear to be a limiting factor in the growth of the suckling rats in these studies. And, as pointed out by Burr and Barnes ('43) one would hardly expect it to do so because of the large storage of linoleic acid and the short experimental period employed. Also supporting the view that linoleic acid is not a limiting factor in this particular case is the fact that feeding ethyl linolate to the young did not improve the rate of gain.

Quackenbush, Kummerow and Steenbock ('42) found that body fats of rats suffering from an essential fatty acid deficiency had higher iodine values than those which had received a supplement of ethyl linolate. In our study the iodine numbers of the body fats of young rats on the low-fat diet were lower than those on the corn oil diet further suggesting that a deficiency of linoleic acid was not a factor limiting growth on the low-fat diet.

Forbes and Swift ('43) have shown that the addition of fat to a diet of protein and carbohydrates decreases the heat increment and thus increases the net energy value of the diet. Such an effect could explain the results observed on the corn oil and the fat-free diet but would offer no explanation of the poor response on hydrogenated coconut oil. It is evident that the kind of fat is an important factor.

SUMMARY

Studies are reported in which the growth of standardized litters to 17 days of age is used as a measure of lactation performance. In these studies rats suckled by mothers fed a diet containing corn oil made more rapid growth than rats whose mothers were fed a fat-free diet. Carcass analyses showed that the extra gain of the young consisted largely of fat.

A diet containing hydrogenated coconut oil gave no better growth than the fat-free diet. No improvement in growth of the young was produced by feeding ethyl linolate to the mothers or directly to the young. Similarly, feeding the mothers 125 mg. of ethyl linolate each day did not improve the lactation response on the fat-free diet.

ACKNOWLEDGMENT

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THE VITAMIN C LEVEL OF THE BLOOD PLASMA IN GUINEA PIGS

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ONE FIGURE

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In the literature on vitamin C determinations in humans there are comments on the inadequacy of making a single, random determination of ascorbic acid in the blood and on the importance of accurately controlling the time elapsing between successive tests for the vitamin in consecutively drawn blood specimens. With the exception of the work of Rohmer et al. ('35) and Zilva ('37), none of the studies on guinea pigs considers these points. Reported normal levels of blood vitamin C values range from approximately 0.0 mg. % to 1.08 mg. %.

It is the purpose of this paper to show that the vitamin C determinations on the blood plasma of guinea pigs are of value only when the time elapsing between the intake of the vitamin and the withdrawal of the blood sample is considered; that the initial value is not a reliable index of the state of vitamin C nutrition of the animal; and that, unless the sampling error is known, a correct interpretation of the experimental data cannot be made.

EXPERIMENTAL

Guinea pigs of both sexes, weighing approximately 250 to 450 gm., were fed for several days before being used, a diet consisting of ad libitum quantities of hay; rolled oats each 100 gm. of which was mixed with 0.25 gm. of sodium chloride, 0.5 gm. of dried brewer's yeast [1] and 1 ml. of U.S.P. XI. cod liver oil; greens, except on Sunday, consisting chiefly of cabbage, celery and carrots; and fresh water.

Ascorbic acid injections were made intraperitoneally in amounts regardless of body weight, since the animals were all within the same weight range.

Blood samples were obtained by lightly anesthetizing the animals with ether and withdrawing 0.5 to 1.0 ml. of blood by cardiac puncture. The survivors were used again after resting for a minimum of 3 days.

[1] Fleischmann.

The determinations of reduced vitamin C were made by the micro method of Farmer and Abt ('34–'35), ('36). A calibrated 0.2 ml. burette graduated in thousandths of a ml. and fitted with a stopcock and a fine capillary was used. Titrations were made with the burette held in a vertical position. Transfers of liquid of 0.2 ml. or less were made with a 0.2 ml. pipette graduated in thousandths of a ml. Determinations were made in triplicate on each sample of plasma. The accuracy of this method was 5.4 ($\pm$ 3.9)%.

In experiment I, plasma vitamin C determinations were made without exact time controls and at different times of day (table 1).

TABLE 1

Random determinations of vitamin C in the blood plasma of normal guinea pigs.

NUMBER OF ANIMALS IN EACH GROUP	TIME OF DETERMINATION AFTER FEEDING GREENS (AD LIBITUM)	PLASMA VITAMIN C MEAN $\pm$ STANDARD DEVIATION
	Hours	*Mg. %*
Group I. (6)	2–3	0.53 $\pm$ 0.26
Group II. (30)	3–6	0.38 $\pm$ 0.15
Group III. (12)	4–6	0.72 $\pm$ 0.51
Group IV. (6)	15–17	0.16 $\pm$ 0.06
Group V. (20)	19–20	0.14 $\pm$ 0.08
Group VI. (32)	42–44	0.16 $\pm$ 0.11
Total (106)	(No greens on Sunday)	

Weighted mean for all animals	0.30 mg. %
Standard deviation	$\pm$ 0.20 mg. %
Standard error	$\pm$ 0.02 mg. %

Experiments II, III, and IV, described below, indicate the rapidity with which the reduced form of the vitamin disappears from the blood.

Experiment II was divided into four parts as follows: (a) Each of nine guinea pigs on the regular diet plus greens ad libitum was injected intraperitoneally with 2.5 mg. of ascorbic acid at 0, 24, 72, and 96 hours. Twenty-four hours after the last injection of ascorbic acid, blood samples were obtained for analysis. On the day of vitamin C determination, no greens were fed. The mean value of reduced vitamin C in the plasma was 0.07 mg. % with a standard deviation of $\pm$ 0.15.

(b) Each of fourteen animals was injected with 5.0 mg. of ascorbic acid at 0, 24, and 48 hours. Blood samples were taken 17 hours after the last injection. No greens were fed on the day of vitamin C determination. The plasma values were 0.06 $\pm$ 0.06 mg. %.

(c) Each of six animals was administered 10.0 mg. of ascorbic acid, at which time all greens were removed. Four hours after the injection, the vitamin C content of the plasma was 0.51 $\pm$ 0.46 mg. %.

(d) Six animals were fed greens at 9:45 A. M.; no injections were given these animals. Two hours later, a blood sample was drawn, and all greens were removed from the cages. The value for this sample was 0.32 ± 0.12 mg. %. Five hours after the withdrawal of the greens, the mean value was 0.08 ±0.04 mg. %.

In experiment III, each of nineteen guinea pigs was injected with 10.0 mg. of ascorbic acid. At the end of 1, 3, and 7 hours after the injection, each of the animals was bled, and the reduced vitamin C content of the plasma determined. A similar experiment was conducted with eighteen animals for the time intervals of ½, 1½, and 4 hours. The results appear in figure 1.

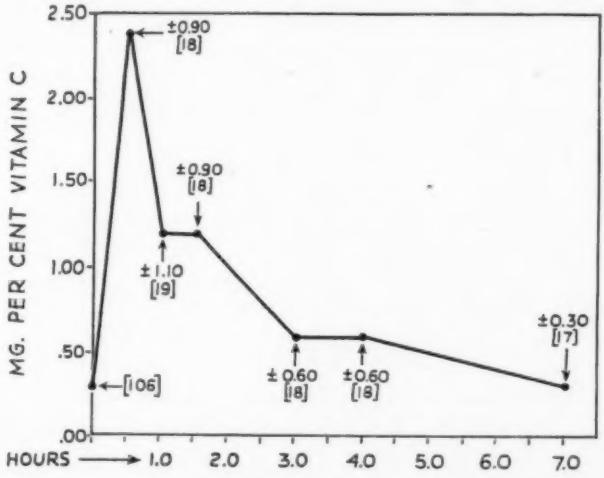

Fig. 1 Rate of disappearance of blood plasma vitamin C in guinea pigs following an intraperitoneal injection of 10.0 mg. of ascorbic acid. Numbers in parentheses represent the number of animals on each determination. Numbers indicated by arrows are the standard deviations for the respective plotted values on the graph.

In experiment IV, the effect of 20 hours of starvation was determined. Twelve guinea pigs were used. The initial plasma vitamin C level was 0.72 ± 0.51 mg. %. After starvation, the value was 0.12 ± 0.07 mg. %. On May 29th, the experiment was repeated with twenty guinea pigs. The initial value was 0.14 ± 0.08 mg. %. After the 20 hours of starvation, the level had dropped to 0.01 ± 0.03 mg. %. For all thirty-two animals before starvation, the weighted mean value was 0.36 ± 0.24 mg. %; after 20 hours of starvation, 0.05 ± 0.05 mg. %. This is a reduction of over 86%.

DISCUSSION

It is apparent that the reduced vitamin C content of the plasma of guinea pigs depends on the time elapsing between the consumption of vitamin C and the withdrawal of the blood sample for the vitamin determination. In figure 1, for example, there is a significant difference of means between the values for ½ hour and 1 hour, between the values for 1 hour and 3 hours, and between those for 3 hours and 7 hours when "t" is greater than 2.00 (Fisher, '34).

From tables 1 and 2, it is obvious that the individual variation in the vitamin C blood plasma levels of guinea pigs is large.

TABLE 2

Significant difference of means for values given in table 1.[1]

GROUP	I 0.53 MG.	II 0.38 MG.	III 0.72 MG.	IV 0.16 MG.	V 0.14 MG.	VI 0.16 MG.
	%	%	%	%	%	%
I 0.53 mg. %	...	1.15	1.06	*3.48*	*3.60*	*3.36*
II 0.38 mg. %	1.15	...	2.00	*2.34*	*2.72*	*2.48*
III 0.72 mg. %	1.06	2.00	...	*3.78*	*3.94*	*3.80*
IV 0.16 mg. %	*3.48*	*2.34*	*3.78*	...	0.63	0.00
V 0.14 mg. %	*3.60*	*2.72*	*3.94*	0.63	...	0.76
VI 0.16 mg. %	*3.36*	*2.48*	*3.80*	0.00	0.76	...

[1] Any figure in italics is considered to indicate a significant difference, since this figure gives a probability of not less than 1 in 20 that the difference would arise by chance.

CONCLUSION

It is concluded, therefore, that a single, random determination of the reduced ascorbic acid content of the plasma of guinea pigs is not indicative of the state of vitamin C nutrition of the animal. In the determination of the state of vitamin C saturation or depletion of guinea pigs, the individual variation and the time elapsing between the last intake of the vitamin and the withdrawal of the blood sample from the animal should be considered.

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FURTHER STUDIES ON THE VITAMIN C METABOLISM OF PRESCHOOL CHILDREN

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The data presented here are part of a larger study on the influence of changes in the ascorbic acid and citrate content of the diet on the ascorbic acid, citric acid, calcium, phosphorus, and nitrogen metabolism of preschool children. Data on the minimum amount of ascorbic acid required to maintain tissue saturation in four preschool children have been published previously (Hathaway and Meyer, '41). The present report is an extension of this earlier work on vitamin C metabolism using eight more children, and also furnishes data on the effect of supplements of ascorbic acid, potassium citrate, and orange juice on the excretion and apparent utilization of ascorbic acid.[2] The effect of these supplements on calcium, phosphorus, nitrogen and citric acid metabolism will be reported separately.

EXPERIMENTAL

Plan of the experiment

Two groups of four children each lived at the college laboratory-apartment for 5 months. During a preliminary period of about a week they became adjusted to their new environment, and the amount of food each child could easily consume was determined. Standardized amounts of the basal foods were consumed not only for each 7-day period, but also for the entire 20 weeks of the experiment. Supplements of crystalline ascorbic acid, potassium citrate, and orange juice were given as indicated in table 1. The amount of potassium citrate used, 3.38 gm., corresponded to the amount of citric acid and its salts calculated to be present in 200 ml. of orange juice, i.e., the amount of orange juice which is needed to supply 100 mg. of ascorbic acid.

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[2] A detailed report of this study is given in the dissertation submitted by Frieda L. Meyer in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of Cornell University, October, 1943.

TABLE 1

Average daily ascorbic acid intakes and "utilization" values by periods for eight preschool children.

1940–41							1941–42						
PERIOD	INTAKE		"UTILIZATION"				PERIOD	INTAKE		"UTILIZATION"			
	Diet	Supplement	A	B	C	D		Diet	Supplement	E	F	G	H
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*		*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
1	23	100	38	34	33	39	1	14	100	35	34	24	26
2	24	100	29	32	30	31	2	15	100	38	36	32	29
3	25	100	30	29	30	30	.	..	...	..	..	..	..
4	23	...	14	14	13	15	3	25[1]	...	18	17	17	17
5	24	...	18	18	19	19	4	24[1]	...	19	18	19	18
6	24	...	18	17	18	19	.	..	...	..	..	..	..
7	22	100	38	37	34	38	5	25[1]	100	34	31	28	25
8	24	100	41	38	46	40	6	25[2]	100	32	29	28	22
9	24	100	47	36	34	37	.	..	...	..	..	..	..
10	24	...	15	16	16	18	7	25[1]	...	17	17	18	16
11	22	...	16	17	17	18	8	25[1]	...	19	19	19	18
12	24	...	19	20	20	20	9	25[1]	...	20	20	20	18
13	24	100	39	36	40	44	10	26[1]	100	37	37	36	32
14	21	100	37	35	38	40	11	26[1]	100	35	33	34	26
..	..	...	..	..	..	..	12	28[1]	100	34	29	32	25
15[2]	22	...	16	15	16	15	13[2]	28[1]	...	21	23	22	20
16[2]	24	...	19	18	19	19	14[2]	27[1]	...	22	23	23	21
17[2]	22	...	18	17	18	19	15[2]	26[1]	...	21	22	22	20
18[3]	23	100	41	34	41	37	16[2]	27[1]	100	49	44	38	34
19[3]	23	100	35	33	37	39	17[2]	27[1]	100	38	35	31	28
20[3]	24	100	41	39	39	40	18[2]	26[1]	100	41	39	36	33
..	..	...	..	..	..	..	19[3]	16	110	43	41	36	34
..	..	...	..	..	..	..	20[3]	17	110	48	41	38	33

[1] Includes 10 mg. crystalline ascorbic acid given daily to compensate for lower ascorbic acid value of basal foods.

[2] 3.38 gm. of potassium citrate given daily as the estimated citrate equivalent of 200 ml. of orange juice.

[3] All ascorbic acid and citrate supplements given in the form of orange juice.

Subjects

Eight healthy preschool children were the subjects for this study. Subjects A through D served in 1940–41, which will hereafter be referred to as the first year, and E through H served in the second year, 1941–42. Subjects A, C, F, G, and H were girls, aged 55, 49, 49, 40, and 38 months, respectively, at the beginning of the study. Their respective weights at that time were 50, 38, 40, 32, and 29 pounds. Subjects B, D, and E were boys, aged 51, 44, and 55 months, weighing 36, 35,

and 34 pounds, respectively. Physical examinations and mental tests at the beginning and end of the study showed that all eight of the children were within the normal range as judged by present standards of measurement.

Diets

The basal foods were similar to those described by Hathaway and Meyer ('41), but minor alterations were made such as changes in the cereals and desserts.[2] In order to test the effect of changes in the ascorbic acid and citrate content of the diet at different levels of calcium intake, the milk consumption was reduced from 800 ml. per day during the first year to 500 ml. the second year. To maintain relatively constant values of the other essential constituents of the diet, the amounts of meat and eggs were increased, and peanut butter was added. The basal foods were analyzed for ascorbic acid and the values were comparable for the 2 years except for the canned potatoes. The same brand contained 18 mg. per 100 gm. the first year, and only 4 mg. the second year. To compensate for this, and also for the lowered ascorbic acid in the milk due to lowered intake,[3] 10 mg. of crystalline ascorbic acid were added to the basal diet during the second year.

The two younger children, G and H, were given somewhat less of the basal diet than the others, but this had little effect on their ascorbic acid intake. Milk and all supplements were given at the same level to all four children in each group.[3]

Ascorbic acid determinations

Methods of urine collection and preservation and of ascorbic acid determination were essentially the same as those described previously (Hathaway and Meyer, '41). The formula suggested by Kuether and Roe ('41) was used for the calculations during the second year, permitting simplification of the determinations of the "Gb" values.

RESULTS AND DISCUSSION

Reserves of ascorbic acid

The response to the 100 mg. supplement of ascorbic acid following periods on the basal diet was used as the means of estimating the extent of saturation of the tissues with this vitamin (see table 2). Bau-

[2] See footnote, page 93.

[3] Specially prepared by Prof. E. S. Guthrie to preserve the ascorbic acid content. See Sharp, Hand and Guthrie ('39).

mann and Rappolt ('37) considered the excretion of at least 80% of a 100-mg. test dose within 24 hours as indicative of tissue saturation. In this study 80% of the 100-mg. test dose was equivalent to 64% of the total ascorbic acid intake. None of the subjects of this study excreted more than 37% of the intake on the first day. Subjects B, E, G, and H excreted 60 to 73% in at least one test by the second day, but only H excreted over 64% in 48 hours in both tests.

TABLE 2

Percentage of ascorbic acid excreted by the eight preschool children on the 100-mg. supplement.

		1940–41						1941–42			
SUBJECT		A	B	C	D	SUBJECT		E	F	G	H
		%	%	%	%			%	%	%	%
On days for adjustment [1]						On days for adjustment [1]					
Day	*Period*					*Day*	*Period*				
1st	7th	14	30	20	24	1st	5th	27	34	31	37
	13th	17	22	9	15		10th	10	15	19	28
2nd	7th	46	60	52	55	2nd	5th	55	41	55	72
	13th	52	63	38	49		10th	64	58	69	73
3rd	7th	64	66	67	66	3rd	5th	72	70	75	76
	13th	58	67	62	67		10th	63	67	71	70
4th	7th	73	72	76	76	4th	5th	84	73	74	77
	13th	67	70	66	69		10th	84	71	69	74
On days after adjustment [2]						On days after adjustment [2]					
Mean		70	72	71	69	Mean		73	75	75	79
Range		(53–81)	(64–79)	(50–84)	(55–82)	Range		(62–84)	(68–86)	(67–83)	(70–87)

[1] Days immediately following periods on the basal diet only.

[2] All but the first two days on the 100 mg. ascorbic acid supplement.

Since 125 mg. of ascorbic acid greatly exceeds the allowance recommended for children of this age range, the excretion value exhibited after adjustment to this intake may be used as a criterion for saturation. The excretion values for these eight children after such adjustment ranged from 50 to 87%. This adjustment required at least 2 days for any child if his lowest excretion value after adjustment is used as the criterion, and 3 to 4 days if his mean excretion value is used.

Further evidence that the tissues were probably not saturated when the subjects were on the basal diet alone is given by comparison of the "utilization" [4] values (table 1) with results from the previous

[4] The term "utilization" is used arbitrarily to refer to the difference between intake and excretion.

study (Hathaway and Meyer, '41; table 4). In the earlier study it was shown that the lowest intake which would maintain tissue saturation was 31 mg. and on this level of intake the "utilization" values averaged 22 mg. The intake on the basal diet in this study was only 23 to 25 mg. and the "utilization" values averaged 18 mg. Since the highest average per cent "utilization" found in any of the twelve children was 80%, one should not expect 22 mg. to be utilized on a 23- to 25-mg. intake. Consequently it may be concluded, using any of these criteria, that the tissues were not completely saturated. The 23- to 25-mg. intake, however, was not far below the amount needed for tissue saturation.

TABLE 3

Means of ascorbic acid "utilization" values and corresponding urinary pH values.

SUBJECT	"UTILIZATION" ON BASAL DIET	pH	"UTILIZATION" ON BASAL DIET PLUS POTASSIUM CITRATE	pH	"UTILIZATION" ON 100-MG. SUPPLEMENT	pH	"UTILIZATION" 100-MG. SUPPLEMENT PLUS POTASSIUM CITRATE	pH
			1940–41					
	mg.		*mg.*		*mg.*		*mg.*	
A	17 ± 2.0	5.7	18 ± 1.1	6.8	37 ± 8.0	5.8	39 ± 7.3	6.8
B	17 ± 1.9	6.0	17 ± 1.5	6.8	35 ± 5.0	5.9	35 ± 6.0	6.8
C	18 ± 2.2	5.7	18 ± 1.7	6.7	35 ± 7.6	5.7	39 ± 8.8	6.7
D	18 ± 1.6	5.6	18 ± 2.0	6.7	37 ± 7.6	5.6	39 ± 5.3	6.7
			1941–42					
E	19 ± 1.6	5.8	21 ± 1.2	6.8	34 ± 6.4	5.7	42 ± 6.6	6.8
F	18 ± 1.7	5.9	22 ± 1.3	6.9	32 ± 6.7	5.9	39 ± 7.2	6.8
G	18 ± 1.5	5.7	22 ± 1.3	6.9	32 ± 6.1	5.6	35 ± 6.6	6.9
H	17 ± 1.5	5.6	20 ± 1.1	6.9	26 ± 6.5	5.5	31 ± 4.7	6.9

The effect of potassium citrate on the "utilization" of ascorbic acid

The mean "utilization" values with the standard deviations are given in table 3. On the basal diet alone the eight children had similar "utilization" values. When potassium citrate was added, the individual responses of the children in the first group were significantly increased in only two cases, subject A on the basal diet, and subject C on the 100-mg. ascorbic acid supplement. With the second group of children, however, the increased "utilization" of ascorbic acid with the addition of potassium citrate was highly significant in every case except G when she was on the 100-mg. ascorbic acid supplement. When the data for the first group were treated by analysis of variance there

was no significant difference in "utilization" between the periods with and without the potassium citrate supplement, but with the data from the second group the odds were 99:1 against the chance occurrence of so great an increase in "utilization."

There is no apparent explanation for the difference in response between the two groups of children. Changes in urinary pH cannot account for the difference, since the increase in pH due to the administration of potassium citrate was of the same order in all the subjects (see table 3).

It is possible that changes in the basal foods might account for the difference in response to citrates. Although the percentage of protein in the diet was the same for both groups, the sources of the protein were different. The decrease in milk and the increase in meat and eggs the second year resulted in the following changes (calculated values) in essential food factors of the basal diet: the calcium was reduced from 1.1 to 0.8 gm., the riboflavin from 1.8 to 1.3 mg. and the acid: base ratio from 1: 1.8 to 1: 1.2.

Purinton and Schuck ('43) have suggested that there may be a relationship between ascorbic acid and citric acid metabolism. The urinary excretion of citric acid in children E, F, G, and H was determined by Metcalf ('43). Changes in citric acid excretion were associated with changes in urinary pH rather than with changes in ascorbic acid excretion.

From the data on these two groups of children there is some indication that addition of potassium citrate to the diet causes an increase in "utilization" of ascorbic acid. The individual differences in response in the first group of children were not mathematically significant in most cases, but when potassium citrate was added to the diet the "utilization" of ascorbic acid was equal to or greater than the "utilization" without citrate in all cases. There were apparently other factors than citrate administration or changes in urinary pH which affected the "utilization" of ascorbic acid. Changes in the high protein foods of the basal diet may have been a factor, but differences in metabolic reactions of the individual children seem more important.

The effect of orange juice on the "utilization" of ascorbic acid

Average ascorbic acid "utilization" values for the two periods during the second year when orange juice was used as a supplement to the basal diet are included in table 1. Higher "utilization" was found for all four children. When the data for the "utilization" values on the

crystalline ascorbic acid supplement and the orange juice supplement were treated by analysis of variance the odds were greater than 99:1 against the chance occurrence of so great an increase in "utilization". When the corresponding data on the ascorbic acid plus potassium citrate supplement were compared with those on the orange juice supplement, the odds were still greater than 19:1 that the ingestion of orange juice was associated with greater "utilization" of ascorbic acid.

The average pH values of the urine during the periods on the orange juice supplement for the four children ranged from 6.0 to 6.2. These values were higher than the corresponding values during the periods on crystalline ascorbic acid (5.5 to 5.9), but were lower than for the periods on potassium citrate (6.8 to 6.9). Thus changes in "utilization" values did not vary directly with the pH changes.

Studies to compare the "utilization" of ascorbic acid in foods with the "utilization" of equivalent amounts of crystalline ascorbic acid are always complicated by the addition of other food factors than those to be tested. Jacobsen ('35), and Hawley et al. ('37) found better storage of ascorbic acid in the tissues when it was administered as a food than when it was given in the crystalline form. Todhunter, Robbins, Ivy, and Brewer ('40) and Todhunter and Fatzer ('40), on the other hand, concluded from both animal and human studies that the response to both forms of ascorbic acid was the same.

Although there was a significant increase in the "utilization" of ascorbic acid in these four children when orange juice was the source of the vitamin, it should not be concluded that the ascorbic acid per se was better utilized until this observation is confirmed by further studies. In these same children potassium citrate, also a constituent of orange juice, improved the "utilization". It may have been an individual response to the potassium ion, to the citrate ion, to changes in acid-base balance, or to some unrecognized food factor which was responsible for the improved "utilization" on the orange juice supplement rather than a difference in the natural and crystalline ascorbic acid. Since the "utilization" values on the orange juice supplement were significantly higher than with potassium citrate added to crystalline ascorbic acid, however, it seems probable that there was some other factor than potassium citrate which was at least partially responsible for the increased "utilization".

SUMMARY

The ascorbic acid metabolism has been studied in eight preschool children. The basal diet containing 23 to 25 mg. ascorbic acid was

supplemented with 100 mg. ascorbic acid, 3.38 gm. potassium citrate, or equivalent amounts of orange juice as indicated. The results were as follows:

1. A daily intake of 23 to 25 mg. of ascorbic acid was not sufficient to maintain tissue saturation in the eight children.

2. On the addition of 3.38 gm. potassium citrate to the diet "utilization" of ascorbic acid was increased in five of the eight children at both levels of ascorbic acid intake (25 and 125 mg.), and in two others at the higher level of ascorbic acid intake.

3. On the substitution of orange juice for crystalline ascorbic acid and potassium citrate, the "utilization" of ascorbic acid was significantly increased in the four children studied.

4. The "utilization" of ascorbic acid was not directly related to the urinary pH.

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THE NUTRITIVE VALUE OF CANNED FOODS

I. INTRODUCTION AND SAMPLING PROCEDURE

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The studies of Kohman and Eddy ('37), sponsored by the National Canners Association during the period 1924–1937, produced information on the nutritive value of canned foods as determined mainly by animal assay methods. Subsequently, further fractionation of the vitamin B complex into well established entities, together with the development of improved methods for their determination and more suitable methods for the determination of other vitamins, emphasized the need for further work. This need was further highlighted by the National Nutrition Conference for Defense in May, 1941. At that time available critical reviews (Daniel and Munsell, '37) contained only limited information on the nutritive values of canned foods. It was obvious that the focus of attention on the nutritional problems of our Armed Forces and civilian population would create new demands for accurate and extensive information on all types of food products, canned foods included.

In recognition of this important need, a nutrition program sponsored jointly by the National Canners Association and the Can Manufacturers Institute was organized in early 1942. This program was administered by an Executive Committee, later assisted by an Industry Advisory Committee. A program of investigation requiring several years for completion was laid out. In broad aspect, this program included: complete information on the nutritive value of commercially canned foods; the effects of preparation methods used in the home, restaurant, and Army kitchens; and the effects of time and temperature of storage on certain vitamins in canned foods. Further, projects were outlined in which the influence of specific canning operations on vitamins in raw foods were to be studied to determine where improvements in existing canning practices might possibly be made.

The 1942 program included the assay of 823 samples of commercially canned foods representing thirty-two products for the factors, ascorbic acid, carotene, riboflavin, niacin, thiamine, and pantothenic acid. Grants

were allocated to various universities where the vitamin assays were carried out under the guidance of authorities in nutritional studies. By correspondence and conference, the methods of assay for the factors were discussed and in all instances the chemical or microbiological methods which were considered to be most applicable were selected. The necessity for correlation studies in which the chemical and microbiological methods could be correlated with officially accepted methods of animal assay was obvious. Arrangements were made to include such work in the first year's program. Studies on the effects of home and large scale kitchen practices (Army and Navy) on the vitamin content of canned foods were initiated. Preliminary investigation was also outlined on the effect of storage on the vitamin content of canned foods. Proximate and mineral analyses (calcium, phosphorus, and iron) of a representative number of the samples of each product were undertaken. Specifically, the 1942 program required the coordinated cooperation of the Universities of Wisconsin, Texas, Chicago, Arizona, Maryland, and Pennsylvania State College. Portions of the program carried out by these institutions under grants-in-aid will be disclosed in subsequent publications.

In consideration of the products which should be assayed during the first year of this program, it was decided that, as a general principle, only non-formulated canned products of nutritional significance, which were produced and consumed in relatively large quantities, should be included. Thirty-two products were selected for the first year's study, including one formulated product, baked beans, because of the Army's interest in it. Samples of these products were taken mainly from the 1942 packs, and, in a few instances, from the spring packs of 1943. The sampling program was organized so that samples of the products in retail size cans (mainly no. 2 and no. 2½ sizes) and of some products in no. 10 cans would be taken from all of the principal canning areas for each specific product. Two or three sampling periods, mid-early and mid-late, or early, middle, and late season samplings, respectively, were planned when seasonal variations in the products might be expected.

The samples were, in most instances, taken from canneries by representatives of the industry designated to collect the samples. Every attempt was made to obtain "run of the mill" samples, in order to provide as good indications as possible of the nutritive value of the major products canned in the United States. It was felt that maximum and minimum vitamin values obtained on a large number of samples of the same product would serve to fix more closely the ranges of vitamin

content for the specific products as well as increase the reliability of the figures for average vitamin contents. In table 1 are shown both the products selected and the scope of sampling.

One sampling at a plant for one period of the season consisted of thirty-six retail size cans or six to twelve no. 10 cans. Where samplings of 46-ounce cans were taken, eighteen cans comprised a sampling. A sampling represented a day's pack of a product in one can size, and, in most instances, consisted of an equal number of cans from the early, middle, and late portion of a day's pack. A complete history of the samples was obtained at the time of sampling, which included all of the information possible about the canned product, raw product used, and the canning procedure employed. It was believed that an analysis of the vitamin and historical data on the samples would reveal some trends in the results which might be of value in future studies of vitamin retention.

The samples were sent to a central sample receiving and distribution point, where they were coded for proper identification, inspected, and divided for shipment to the various laboratories. Two retail size cans or one no. 10 can from each sampling were checked for vacuum and headspace. Net weight, drained weight, pH and general quality characteristics of the products were determined. Six retail size cans or one no. 10 can from each sampling were sent to each university conducting the vitamin assays. Some of the samples of certain products in retail and no. 10 can sizes were used for study of the distribution of water soluble nutrients between the solid and liquid portions of the can contents, and for studies on the effect of preparation for serving on the vitamin content. The remaining samples were held in storage as duplicate samples, some being used for the proximate and mineral analyses.

The six retail size cans of each sampling received by each collaborator were combined into one composite sample and representative portions taken for the assay of two vitamin factors. Thus each analysis of a sampling of the retail size cans included a sufficient number of cans and quantity of product to give a good indication of the vitamin values on a day's pack at a given plant. When more than one sampling of no. 10 cans of a product were taken at one plant during the season, two or three no. 10 cans, one from each sampling, were combined into one composite sample.

The vitamin values obtained by the collaborators on the canned samples are reported in the accompanying papers. The reports on the other work undertaken in this program will appear in subsequent publications.

TABLE 1

Products collected from 1942 packs for determination of nutritive value and composition.

PRODUCT	NO. OF PLANTS SAMPLED	EASTERN DISTRICT				MIDWESTERN DISTRICT				WESTERN DISTRICT			
		Retail size cans		No. 10 cans		Retail size cans		No. 10 cans		Retail size cans		No. 10 cans	
		No. of plants	No. of samples	No. of plants	No. of samples	No. of plants	No. of samples	No. of plants	No. of samples	No. of plants	No. of samples	No. of plants	No. of samples
Apricots, unpeeled halves	8	..	..	..	..					13	21		
Asparagus, all green	15	3	7	2	2	6	6	4	4	5	7	5	5
Asparagus, culturally bleached	4	..	..	..	..	..	..	..	..	4	8	4	4
Beans, baked New England style	3	..	..	2	2	..	..	..	..	..	..	1	1
Beans, baked with tomato sauce	2	..	..	1	1	..	..	1	1	..	..	..	..
Beans, green cut	23	3	9	3	3	10	29	10	10	6	16	6	6
Beans, lima, green	14	11	13	7	7	3	5	2	2	..	..	..	..
Beets	9	3	6	3	3	4	8	4	4	2	4	2	2
Carrots	7	2	4	2	2	3	6	3	3	2	2	2	2
Corn, white, whole kernel	15	4	12	2	2	10	28	4	4	..	..	..	..
Corn, yellow, whole kernel	24	3	9	3	3	12	31	9	9	2	6	2	2
Grapefruit juice	20	8	18	..	..	9	18	..	..	3	7	..	..
Grapefruit segments	10	8	19	..	..	2	5	..	..	..	..	..	..
Mackerel	8	2	3	..	..	..	..	..	..	6	6	..	..
Orange juice	8	3	4	..	..	..	..	..	..	5	11	..	..
Peaches, halves (clingstone)	9	..	..	..	..	..	..	..	..	9	17	..	..
Peaches, halves (freestone)	11	1	2	..	..	1	2	..	..	9	9	..	..
Pears, halves	15	1	2	..	..	2	4	..	..	12	24	..	..
Peas, sweet (wrinkled varieties)	26	6	18	4	4	11	31	10	10	8	24	5	5
Peas, Alaska	2	..	..	..	..	2	6	1	1	..	..	..	..
Pineapple juice	8	..	..	..	..	..	..	..	..	8	18	..	..
Pineapple, sliced	8	..	..	..	..	..	..	..	..	8	17	..	..
Prunes, Italian	5	..	..	..	..	..	..	..	..	5	10	..	..
Salmon	5	..	..	..	..	..	..	..	..	5	5	..	..
Sardines, in oil	5	5	5	..	..	..	..	..	..	..	..	..	..
Sardines, in tomato sauce	10	..	..	..	..	..	..	..	..	10	10	..	..
Shrimp, dry pack	3	3	3	..	..	..	..	..	..	..	..	..	..
Shrimp, wet pack	5	5	5	..	..	..	..	..	..	..	..	..	..
Spinach	19	3	3	2	2	5	5	2	2	11	12	7	7
Tomatoes	26	6	17	..	..	7	18	1	1	10	23	2	4
Tomato juice	30	12	35	..	..	8	23	..	..	10	21	..	..
Tuna	6	..	..	..	..	..	..	..	..	6	6	..	..
Total	363	92	194	31	31	95	225	51	51	159	284	36	38

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THE NUTRITIVE VALUE OF CANNED FOODS

II. ASCORBIC ACID AND CAROTENE OR VITAMIN A CONTENT [1]

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For intelligent planning, evaluation, and rationing of human dietaries, information concerning the nutritive value of all varieties of foods grown, prepared or processed under different conditions is greatly needed. It is the purpose of this paper to report the findings of the ascorbic acid and carotene or vitamin A assays of certain commercially canned fruits, vegetables, and fish.

EXPERIMENTAL

Details of the method of the collection of samples of the foods used in this study, which were grown, processed, and canned in different sections of the country and at different seasons of the year have been presented in a preceding paper in this journal (Clifcorn, '44). Information concerning the various sizes of the cans and the quantity of each bearing the same code numbers, which were selected to be composited for vitamin assay, has also been given. Ascorbic acid and carotene or vitamin A analyses of 814 composited samples of thirty-three different canned foods were begun in October, 1942, and completed in July, 1943. During this period, the canned foods were stored on the shelves of a laboratory storeroom at ordinary room temperatures, ranging from 20 to 31° C.

Sampling [2]

As soon as the canned foods were opened the liquids were drained from the solids into glass beakers, and the solid and liquid portions of the canned contents weighed separately. Composited semi-homogeneous samples of the combined solid and liquid contents of all the

[1] This work was financed and planned by the National Canners Association — Can Manufacturers Institute Nutrition Program (see Clifcorn, '44).

[2] Following the plan of the National Canners Association — Can Manufacturers Institute Project, vitamin assays were made upon samples of the combined solids and liquids of the canned foods.

cans in the same group were then obtained by blending the total can contents in the Waring Blendor from 15 seconds to 2 minutes, the time depending upon the character of the material. Suitable aliquots of the comminuted material were weighed immediately and prepared for analysis.

Analytical methods

The vitamin assay methods selected for use in this study are among those recommended by the Committee on Vitamin Assay Methods of the National Cooperative Experiment Station Project on Conservation of the Nutritive Values of Foods.

Vitamin C. The ascorbic acid content of all the canned foods was determined following the chemical method of Bessey ('38) and modified by Morrell ('41) briefly described as follows: Twenty-five gram samples, of the comminuted solids and liquids or 25 ml. of the canned juices (tomato, grapefruit, orange, and pineapple) were placed in 100 ml. volumetric flasks and brought up to volume with 3% metaphosphoric acid solution. In general, the contents of the flasks were thoroughly shaken and then filtered through dry, fluted Whatman no. 12 filter paper. Only in the case of corn which, because of the toughness of its hull, was found more difficult to homogenize, did better extraction of the ascorbic acid result from the use of the Waring Blendor. Separation of the solid material of corn was also better accomplished by centrifuging.

Aliquots of the almost clear filtrate containing from 100 to 200 μg. of ascorbic acid were transferred to 50-ml. volumetric flasks and their pH adjusted to approximately 3.6 with a sodium citrate buffer. This mixture was then brought up to volume and to a final pH of 3.6 $\pm$ 0.1 with a sodium hydroxide, citric acid, and metaphosphoric acid buffer solution. Suitable size of the aliquots selected for the different canned foods was found to range from 1 to 2 ml. for the citrus fruits; 3 to 5 ml. for asparagus, spinach, and tomatoes; 5 ml. for peas, pineapple juice, and lima beans; and 10 to 15 ml. for peaches, pears, and most of the other canned foods of low vitamin C value. The bleaching effect of 5 ml. of this solution on 5 ml. of a solution of the dye 2, 6-dichlorobenzenoneindophenol (20 mg. per liter of water) was then measured, following in detail the Morell ('41) method using the Evelyn Photoelectric Colorimeter with the green filter no. 520. Correction was made for interference caused by the turbidity and the color of the unknown test solutions in the usual manner. In the case of beets and prunes, the size of the aliquot used was necessarily limited to 5 ml. in spite of their low ascorbic acid content because of the excessive in-

terference caused by the deepness of their color. Only in the case of asparagus was there observed a rapid rate of fading and therefore a high rate of drift in the galvanometer readings after the addition of the dye solution. When correction was made for this by subtraction of the difference between the 15-second and the 30-second readings as proposed by Bessey ('38), duplicate samples checked within limits of the accuracy of the method.

The colorimeter readings were interpreted from calibration curves, made from standard solutions of pure ascorbic acid.

Vitamin A. The vitamin A value of all the canned fruits and vegetables was measured by their pro-vitamin A or carotene content following the chemical assay procedure of Moore ('40) and modified by Moore and Ely ('41). Briefly described, the carotene in comminuted solid and liquid samples was extracted in a mixture of approximately 57% of 95% ethyl alcohol and 43% of a petroleum ether solution [3] by blending for 5 minutes in a Waring Blendor. The two liquids in the mixture were then completely separated in a separatory funnel by the addition, if necessary, of enough water to make the alcohol concentration about 80% by volume. The alcoholic phase was then drawn off and the residue washed three or more times with 30-ml. portions of Skellysolve B. The combined extract was subsequently washed six times with tap water in order to remove all of the alcohol and then made up or reduced to convenient volume as indicated by depth of the color. Pigments other than carotene were removed by passing the carotene extracts through tubes containing dicalcium phosphate as the absorbent material. The filtrate was then concentrated if necessary in a hot water bath (70° C.) under reduced pressure and made up to a suitable volume. The carotene content was then measured using the Evelyn Photoelectric Colorimeter with filter no. 440. Readings were interpreted from a previously prepared calibration curve based on standard solutions of pure beta-carotene dissolved in Skellysolve B. A standard solution of potassium dichromate was used periodically to check the calibration of the colorimeter.

In this study the size of the sample selected for analysis which was found to be most convenient for several reasons was as follows: 10 gm. of foods of high carotene content, carrots, apricots, and spinach; 30 to 35 gm. of foods very low in carotene content, such as baked beans, beets, pineapple, grapefruit, orange juice, pears, and lima beans, etc.; and 15 to 25 gm. of all the other fruits and vegetables, all being weighed

[3] Skellysolve B, Skelly Oil Company, Chicago, Illinois.

to 0.01 gm. When even large samples of the foods very low in carotene content were extracted it was found necessary to evaporate the extracted solutions almost to dryness. The entire extract was then passed through the dicalcium phosphate column by suction in 4 to 5 minutes, washed with Skellysolve B and brought up to 10- to 25-ml. volumes, in order to have colorimeter readings fall within the most sensitive portion of the calibration curve. However, the samples of most of the foods taken were large enough to yield extracts sufficiently rich in carotene to make it unnecessary to reduce the volume by evaporation to such a great extent. Instead, suitable aliquots of the extract rather than the entire amount could be passed through the dicalcium phosphate column. Thus, a saving not only of time but of solvent was effected. It was not necessary to reduce the volumes of the carotene extract of apricots, carrots, or spinach but instead they were increased to a volume of 200 to 250 ml. and 10-ml. aliquots taken for analysis. Volumes of the extracts of most of the other foods were reduced to 50 or 100 ml. from which 20- to 25-ml. aliquots were taken to pass through the absorbent.

In most cases Baker's Analyzed C. P. dicalcium phosphate was found to be a suitable absorbent of the pigments other than carotene and to permit a 98 to 100% recovery of pure beta-carotene. It was found necessary to control the speed of passage of the extracted material by the addition of Dyno [4] in some cases but not in others and to vary the height of the column. For example, the dicalcium phosphate column had to be longer than usual for the complete removal of interfering pigments from corn and peaches and to prevent them from being washed into the filtrate. Care was taken to pack the tubes evenly to prevent channeling. The greatest difficulty of all was encountered in the case of tomatoes because of the incomplete absorption of lycopene when Baker's material was used as the absorbent. The most satisfactory absorption of lycopene was obtained when lots of dicalcium phosphate prepared in this laboratory following the method of Moore ('42) were used. The reason for this is not understood. Only certain batches of this laboratory prepared material were found to be satisfactory. It was noted that the absorbing ability or activity of the product varied with the yield. For example, small yields of a very fine material held back all of the lycopene but also did not permit the passage of the carotene. On the other hand, certain large yields of a very coarse granular material which permitted the passage of carotene also allowed most of the lycopene to pass through. It was also noted that activated di-

[4] Corn Products Refining Company, 17 Battery Place, New York.

calcium phosphate frequently became inactive after a period of time in storage.

The vitamin A in canned fish has been determined following in general the Carr-Price method ('26) as modified by Dann and Evelyn ('38) for use with the Evelyn Photoelectric Colorimeter. Cans of tuna fish, sardines which were packed in oil, and wet pack shrimp were drained and only the solids were used for analysis as directed. Assays of salmon, mackerel, and dry pack shrimp were made on combined can contents. Analyses were made of 20- to 25-gm. portions of the fish paste weighed into Erlenmeyer flasks. Five milliliters of a saturated solution of potassium hydroxide and 20 ml. of methyl alcohol were added for every 10 gm. of fish, and the samples were refluxed for 10 minutes on a water bath and cooled under tap water. The mixture was then transferred to a separatory funnel and the vitamin A extracted by shaking it three times with 50-ml. portions of ethyl ether. The ether fractions were combined and washed with tap water seven to eight times until the wash water was neutral to litmus paper. The extract was then dried by passing through anhydrous sodium sulfate in a sintered glass funnel and evaporated to dryness under reduced pressure. Traces of moisture were expelled by placing the flask in the oven at 100° C. for 30 seconds. The unsaponifiable residue was then transferred and brought up to 10-ml. volume with dry chloroform. In measuring the vitamin A content of the test solution, 1 ml. was placed in the Evelyn colorimeter tube using filter no. 620 and antimony trichloride as the blank, and 9 ml. of antimony trichloride reagent (25 gm. antimony trichloride in 100 ml. chloroform) quickly added. Readings were made as the galvanometer pointer rested briefly before the color began to fade and the needle began to drift. The readings were interpreted by means of a calibration curve made with standard solutions of pure crystalline vitamin A alcohol [5] dissolved in chloroform.

Duplicate determinations were made on all samples of canned fruits and vegetables until the reliability of the method for each food was believed to be established. Great difficulties were encountered in the analyses of fish and the lack of close agreement between duplicates makes the values reported for fish more open to question.

RESULTS

The vitamin assay data summarized as to range and average for each of the different types of fruits and vegetables are presented in table 1.

[5] Distillation Products, Inc., Rochester, New York.

TABLE 1

Ascorbic acid and carotene content of commercially canned fruits and vegetables.

FOODS	ASCORBIC ACID			CAROTENE		
	Number of composite samples analyzed	In 100 gm. combined solids and liquids		Number of composite samples analyzed	In 100 gm. combined solids and liquids	
		Range mg.	Average mg.		Range mg.	Average mg.
Apricots, unpeeled halves	21	1.1– 5.7	3.9	21	0.93–1.74	1.28
Asparagus, all green	30	5.4–25.3	15.2	30	0.22–0.50	0.31
Culturally bleached [1]	12	11.6–18.1	14.9	12	Trace–0.06	0.03
Beans, baked						
New England style	3	1.9– 4.3	2.9	3	Trace–0.05	0.02
With tomato sauce	2	1.8– 3.5	2.7	2	0.04–0.06	0.05
Beans, green cut	73	0.6– 7.3	3.2	73	0.08–0.34	0.18
Beans, lima, green	26	2.5–12.1	7.0	26	0.03–0.18	0.07
Beets	27	Trace–4.0	2.0	27	Trace–0.05	0.006
Carrots	19	1.0– 4.6	2.0	19	4.47–9.44	7.16
Corn, white, whole kernel	45	2.0– 6.5	4.4	28	Trace–0.11	0.02
Yellow, whole kernel	61	1.3– 8.0	4.2	61	0.04–0.16	0.09
Grapefruit juice	43	26.3–44.7	33.8	18	Trace–0.02	0.007
Grapefruit segments [2]	22	20.3–32.3	24.6	3	Trace–0.02	0.009
Orange juice	14	33.0–52.4	39.4	14	0.01–0.12	0.08
Peaches, halves, clingstone	17	2.0– 5.7	3.9	17	0.19–0.51	0.26
Freestone	13	1.4– 3.7	2.3	13	0.09–0.28	0.16
Pears, halves	30	Trace–2.5	1.5	4	Trace–0.002	
Peas, sweet,						
wrinkled varieties	91	3.1–13.8	8.8	91	0.15–0.51	0.26
Alaska	7	7.4–13.7	10.0	7	0.21–0.31	0.25
Pineapple juice	18	3.2–14.2	8.5	18	0.01–0.04	0.03
Pineapple, sliced	17	0.8– 0.7	5.1	17	0.01–0.04	0.03
Prunes, Italian [3]	10	Trace–3.0	1.1	10	0.44–0.89	0.63
Spinach	31	3.4–25.9	11.4	31	1.68–4.81	3.16
Tomatoes [4]	61	9.5–27.1	16.5	61	0.35–0.96	0.58
Tomato juice	79	2.5–25.2	12.9	79	0.16–0.96	0.51

[1] Culturally bleached asparagus is asparagus so cultivated that the development of chlorophyll is inhibited. The color of canned culturaly bleached asparagus may be practically white, yellow-white, or the heads may be yellow-green, green, or purple.

[2] Two cans of "pink" grapefruit omitted because the values were not within the normal range for carotene.

[3] Edible portion, skins and pulp, analyzed.

[4] Seeds included.

Vitamin A is expressed in terms of milligrams of carotene per 100 gm. of combined solids and liquids, or 100 ml. of the canned juices.

Vitamin C is expressed in terms of milligrams of ascorbic acid in 100 gm. of combined solids and liquids, or 100 ml. of the canned juices.

Table 2 presents the results of the vitamin assay of canned fish, expressed in terms of milligrams of pure vitamin A alcohol per 100 gm.

TABLE 2

Vitamin A content of canned fish.

FOODS	NUMBER OF COMPOSITE SAMPLES ANALYZED	VITAMIN A VALUE MG. PER 100 GM.	
		Range	Average
Mackerel [1]	8	0.015–0.054	0.029
Salmon, red species	3	0.078–0.102	0.087
Pink species	2	0.015–0.023	0.019
Sardines, in oil	5	0.021–0.123	0.069
In tomato sauce [2]	10	.....	....
Shrimp, dry pack	3	0.014–0.023	0.017
Wet pack	5	0.015–0.022	0.018
Tuna [1]	6	0.005–0.010	0.008

[1] Oil drained off.

[2] Assays of sardines in tomato sauce not included in report because of unreliability of method of analysis due to interfering pigments.

It may be seen in table 1 that on the basis of their average ascorbic acid content, different kinds of canned fruits and vegetables may be grouped as follows:

Canned orange and grapefruit juices, as expected, are the best sources of vitamin C of the canned foods studied. The average ascorbic acid content was found to be 39.4 mg. per 100 ml. for orange juice and 33.8 mg. per 100 ml. for grapefruit juice. It was found that canned grapefruit segments in sirup were lower in ascorbic acid value, showing an average of 24.6 mg. per 100 gm.

Tomatoes, all green asparagus, culturally bleached asparagus, tomato juice, and spinach were found to lie in the range of 16.5 to 11.4 mg. per 100 gm. or ml.

The average ascorbic acid content of peas (including Alaska's), pineapple juice, and lima beans fell within the range of 8.9 to 7.0 mg. per 100 gm. or 100 ml. of the canned contents. The ascorbic acid content of canned sliced pineapple in sirup was relatively lower than pineapple juice alone, for it was found to yield 5.1 mg. per 100 gm.

White corn, yellow corn, clingstone peaches, apricots, green beans, baked beans, freestone peaches, beets, carrots, pears, and prunes are the poorest sources of vitamin C, yielding in the order named on the average from 4.4 to 1.1 mg. of ascorbic acid per 100 gm. combined solids and liquids.

On the other hand, when considered from the standpoint of their vitamin A value, the same commercially canned goods fell into the following groups:

The best sources of carotene among the canned foods which were tested were carrots, spinach, and apricots. The average carotene content of the combined solid and liquid of these foods was found to be 7.16, 3.16, and 1.28 mg. per 100 gm., respectively.

Prunes (pulp and skins), tomatoes, tomato juice, all green asparagus, peas, and clingstone peaches were found to be lower in carotene and to range on the average from 0.63 to 0.26 mg. per 100 gm. or ml.

Green beans, freestone peaches, yellow corn, orange juice, and lima beans were found to range from 0.18 to 0.07 mg. per 100 gm. Canned red salmon and sardines (table 2) are the only canned fish that showed vitamin A values approximately of this order.

Culturally bleached asparagus, pineapple juice, sliced pineapple, baked beans, and white corn showed a range in average carotene content of 0.03 to 0.02 mg. per 100 gm. or 100 ml.

Grapefruit segments, grapefruit juice, beets, and pears are very poor sources of pro-vitamin A, ranging from 0.009 mg. to a trace per 100 gm. of combined solids and liquids.

Unfortunately, it is common practice for the liquid present in certain types of canned vegetables to be discarded when served. Fortunately, however, from the standpoint of vitamin A value, carotene is not highly water soluble and therefore is retained to a large extent in the food solids. In table 3, therefore, the carotene content of the canned foods, which in table 1 have been shown to be the best sources of this vitamin, has been expressed on the solid basis. This has been

TABLE 3

Carotene content of solid portions of certain canned foods.[1]

FOODS	NUMBER OF COMPOSITE SAMPLES ANALYZED	APPROXIMATE PERCENT OF SOLIDS IN CAN [2]	ESTIMATED CAROTENE CONTENT OF 100 GM. OF SOLIDS	
			Range mg.	Average mg.
Apricots, unpeeled, halves	21	60	1.56– 2.90	2.13
Asparagus, all green	30	64	0.34– 0.78	0.48
Beans, green, cut	73	62	0.13– 0.55	0.30
Beans, lima, green	26	67	0.05– 0.26	0.11
Carrots	19	68	6.57–13.88	10.54
Corn, yellow, whole kernel	61	66	0.05– 0.25	0.14
Peaches, halves, clingstone	17	66	0.29– 0.78	0.39
Peaches, halves, Freestone	13	65	0.13– 0.43	0.24
Peas, sweet, wrinkled varieties	91	65	0.24– 0.78	0.40
Prunes, Italian	10	51	0.86– 1.75	1.23
Spinach	31	69	2.44– 6.97	4.58

[1] Based on the assumption that all carotene is in the solid portion.

[2] Calculated from average weights of solids and liquids in each individual can contents.

estimated on the assumption that all of the carotene is present in the solid and none in the liquid portion. Canned peaches, apricots, and prunes, fruits which are good sources of carotene, have also been included in this table, although it is customary to serve canned fruits with their sirup.

DISCUSSION

The number of composited samples which were assayed for ascorbic acid and carotene ranged for fruit from the minimum of 10 samples of prunes to 65 of grapefruit segments and juice and for vegetables from the minimum of 26 samples of lima beans to 140 samples of tomatoes and tomato juice. It may be noted that the range in ascorbic acid and carotene content of many of these foods is very wide. This difference, of course, is of practical importance only when the food is a good source of the vitamin in question.

Obviously differences in the vitamin values of different samples of any of these canned foods should be expected for the many samples of each kind of food analyzed include different varieties, foods grown under different soil and climatic conditions, in different parts of the country, selected from early, middle, or late parts of the season's pack, processed by different canners, and packed in cans of different sizes with some differences in the ratio of solid to liquid can contents.

It may be noted, for example, that canned clingstone peaches were found to be somewhat better sources of both ascorbic acid and carotene than freestones. Canned red salmon proved to be substantially richer in vitamin A than pink salmon, and again, canned yellow corn was found to be far richer in carotene than white corn, although there was no significant difference in their ascorbic acid content. As expected, culturally bleached asparagus was very much lower in carotene content than the same variety of asparagus so cultivated that the development of chlorophyll was not inhibited (all green). Again, however, there was no significant difference in their ascorbic acid content. The wide range of the average carotene values of canned culturally bleached asparagus (although relatively insignificant because it is a poor source of carotene) appeared to be associated with the variation in the chlorophyll content as indicated by some cans containing green-tipped heads, as contrasted with others in which the color of the asparagus heads was practically white or yellow-white.

Samples of both grapefruit segments and pineapple slices were found lower in ascorbic acid value than the canned juices of these fruits. This is to be expected because of the dilution resulting from the addition of sirup to the fruit packs. On the other hand, the samples of canned tomato juice appeared to be somewhat lower in ascorbic

acid content than the canned tomatoes. Since no diluting medium such as sirup is added in the canning of tomatoes, these differences may perhaps be associated with differences in the canning procedure.

The wide range in both the ascorbic acid and carotene values reported in table 1 for some of the canned foods such as tomatoes and tomato juice, orange juice, spinach, carrots, etc., may perhaps be associated with differences in variety and section of the country in which they were grown and processed.

SUMMARY

Certain commercially canned foods have been assayed for their ascorbic acid and carotene or vitamin A values following recommended chemical methods.

The ascorbic acid and carotene contents of 21 composite samples of canned apricots, 30 all green asparagus, 12 culturally bleached asparagus, 73 green beans, 26 lima beans, 5 baked beans, 27 beets, 19 carrots, 61 yellow corn, 14 orange juice, 17 clingstone peaches, 13 freestone peaches, 98 peas, 17 sliced pineapple, 18 pineapple juice, 10 prunes, 31 spinach, 61 tomatoes, and 79 tomato juice have been determined. The ascorbic acid content of 45 samples of white corn, 22 grapefruit segments, 43 grapefruit juice, and 30 pears, and the carotene content of 28 samples of white corn, 3 grapefruit segments, 18 grapefruit juice, and 4 pears have also been determined.

Eight composite samples of mackerel, 5 salmon, 15 sardines, 8 shrimp, and 6 tuna have been analyzed for vitamin A.

The analytical data for each food have been presented in tabular form as range and average values.

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THE NUTRITIVE VALUE OF CANNED FOODS

III. THIAMINE AND NIACIN [1]

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In an effort to enlarge upon present knowledge of the nutritional value of canned foods the National Canners Association-Can Manufacturers Institute has collected a large number of canned food samples and has distributed these to university laboratories for assay. It is the purpose of this paper to report the results of thiamine and niacin assays on these samples.

EXPERIMENTAL

The collection and distribution of the samples is described in a separate paper (Clifcorn, '44). The same report contains the details of coding, packing dates, collection by areas and can sizes. When received at this laboratory the coded samples were stored in the basement room until assayed. The temperature of the room ranged from 22°C. to 26°C. for the duration of the work, September 1942 to August 1943.

Preparation of samples

The contents of all cans taken for one sample (6 consumer size cans or 1 to 3 no. 10 cans) were combined and mixed. The solids of green beans, lima beans, carrots, white and yellow whole kernel corn, peas, apricots, asparagus, beets, grapefruit segments, peaches, tomatoes, pineapple slices, spinach, and pears were separated from the liquids on a screen. The solids were weighed and the liquid volume determined. One-tenth of the solid and liquid portions (300 to 400 gm. total weight) were recombined, and this reconstituted aliquot was blended with 2–3 ml. of chloroform in a Waring Blendor. In the case of apricots,

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asparagus, beets, grapefruit segments, peaches, tomatoes, pineapple slices, spinach, and pears, the foods were macerated by a propeller-mixer or cut to a small particle size by hand before the solid and liquid portions were separated. Prunes were treated in a similar manner, except that the pits were removed and only the edible portion macerated, blended and analyzed.

The various canned juices (tomato, grapefruit, orange and pineapple) were not homogenized in the Waring Blendor, but the contents of the individual cans were simply stirred together. The desired aliquot was then removed and 2–3 ml. of chloroform added.

Baked beans, salmon, dry-packed shrimp, and sardines in tomato sauce were hand-mixed, and a weighed amount, plus 2–3 ml. of chloroform, was blended with sufficient water to form a thick slurry. Tuna, brine-packed shrimp, sardines in oil, and brine-packed mackerel, however, were drained, the oil or brine discarded, and the solid portion then treated in the same way as the baked beans, salmon, shrimp and sardines.

Assay methods

Thiamine. Ten-gram samples of the blended foods were weighed out for extraction with 0.1 N H_2SO_4 and subsequent analysis. Thiamine was determined by the thiochrome method of the Research Corporation Committee based on the thiochrome method (Hennessy, '42) with the following modifications: (a) extractions were made at 70°C. for 1 hour rather than ½ hour in boiling water; (b) enzymatic digestion was performed at 37°C. over-night rather than 2 hours at 45–50°C.; (c) instead of using a separate series of standards, a standard value was obtained by adding a standard thiamine solution to a third aliquot of the KCl-HCl eluates and determining the increased thiochrome produced over that of the eluate alone.

Niacin. Five grams of the homogenized sample were hydrolyzed with 50 ml. of N NaOH at 15 pounds pressure for 30 minutes. After hydrolysis the suspension was adjusted to a pH of 6.6–6.8 with N HCl (brom thymol blue, outside indicator) and diluted to give an estimated concentration of 0.05 to 0.1 μg. of niacin per milliliter. Fish samples were hydrolyzed with 50 ml. of N H_2SO_4 at 15 pounds pressure for 30 minutes. The sample was then adjusted to a pH of 6.6–6.8 with N NaOH and diluted to the same concentration as mentioned above.

The assay method of Snell and Wright ('41) was employed with the modified medium of Krehl, Strong and Elvehjem ('43). Each sample was set up at five levels (1, 2, 3, 4, and 5 ml. of test suspension per tube), and duplicate assays were run on different days.

RESULTS AND DISCUSSION

The results obtained are summarized in table 1. The values are expressed as milligrams of the vitamin per 100 gm. of the entire original contents of the can except in the cases noted previously. The data presented are believed to be much more extensive than anything heretofore published on the thiamine and niacin content of canned foods. Sufficiently large numbers of samples were analyzed so that the average values found may be considered to represent quite closely the true vitamin content of the foods studied.

TABLE 1

Thiamine and niacin content of canned foods.

PRODUCT	NO. OF SAMPLES	THIAMINE HCl		NIACIN	
		Range	Average	Range	Average
		mg. %	*mg. %*	*mg. %*	*mg. %*
Apricots, unpeeled halves	21	0.008–0.026	0.019	0.20–0.48	0.37
Asparagus, all green	30	0.040–0.122	0.067	0.22–1.19	0.85
Culturally bleached	12	0.043–0.055	0.050	0.63–0.88	0.75
Beans, baked New England style	3	0.015–0.027	0.021	0.15–0.32	0.24
With tomato sauce	2	.....	0.053	.....	0.91
Green cut	75	0.011–0.053	0.029	0.18–0.60	0.32
Lima, green	27	0.019–0.048	0.032	0.32–0.77	0.55
Beets	27	0.004–0.014	0.008	0.06–0.28	0.13
Carrots	19	0.013–0.027	0.021	0.19–0.49	0.34
Corn, white, whole kernel	46	0.010–0.039	0.021	0.46–1.50	0.88
Yellow, whole kernel	61	0.014–0.045	0.026	0.53–1.06	0.77
Grapefruit juice	43	0.012–0.050	0.025	0.08–0.49	0.17
Segments	24	0.007–0.046	0.026	0.13–0.35	0.21
Mackerel	9	0.021–0.045	0.034	4.01–11.4	7.82
Orange juice	13	0.033–0.103	0.072	0.18–0.30	0.25
Peaches, clingstone, halves	17	0.005–0.011	0.007	0.48–1.18	0.70
Freestone, halves	13	0.005–0.011	0.008	0.34–0.90	0.57
Pears, halves	30	0.005–0.012	0.009	0.06–0.28	0.13
Peas, sweet, wrinkled varieties	94	0.056–0.188	0.115	0.42–2.69	1.06
Alaska	6	0.085–0.121	0.099	0.68–1.04	0.80
Pineapple juice	18	0.031–0.070	0.052	0.16–0.20	0.18
Sliced	17	0.053–0.087	0.070	0.12–0.20	0.17
Prunes, Italian	10	0.017–0.039	0.024	0.19–0.47	0.36
Salmon	5	0.014–0.038	0.021	5.95–8.91	7.81
Sardines, in oil	5	0.014–0.042	0.024	2.92–7.15	5.57
In tomato sauce	10	0.007–0.016	0.010	2.36–5.40	3.93
Shrimp, dry pack	3	0.006–0.011	0.009	1.10–3.40	2.23
Wet pack	5	0.004–0.011	0.008	0.72–2.52	1.36
Spinach	31	0.009–0.041	0.020	0.16–0.64	0.30
Tomatoes	63	0.019–0.077	0.049	0.41–0.97	0.69
Tomato juice	77	0.014–0.063	0.049	0.55–1.77	0.75
Tuna	6	0.016–0.082	0.037	7.60–13.0	10.2

The extent of the range in thiamine content varied considerably from product to product. The highest values for apricots, green beans, white corn, grapefruit juice, peas, spinach, tomato juice and tomatoes were approximately four times the lowest values. The range for grapefruit sections and tuna fish was 5- to 6-fold, while that for the remaining foods was 3-fold or less.

TABLE 2

Comparison of can size and vitamin content.

PRODUCT	CAN SIZE	NO. OF SAMPLES	THIAMINE		NIACIN	
			Range	Average	Range	Average
			mg. %	*mg. %*	*mg. %*	*mg. %*
Asparagus, all green	C[1]	20	0.045–0.122	0.071	0.60–1.07	0.89
	I[2]	10	0.040–0.090	0.059	0.22–1.19	0.78
Culturally bleached	C	8	0.044–0.055	0.050	0.64–0.83	0.74
	I	4	0.043–0.052	0.050	0.63–0.88	0.77
Beans, green cut	C	54	0.011–0.053	0.028	0.18–0.60	0.31
	I	21	0.017–0.047	0.031	0.27–0.44	0.32
Lima, green	C	18	0.023–0.048	0.034	0.32–0.77	0.53
	I	9	0.019–0.041	0.027	0.33–0.67	0.58
Beets	C	17	0.004–0.012	0.008	0.06–0.28	0.12
	I	10	0.006–0.014	0.009	0.08–0.22	0.14
Carrots	C	12	0.013–0.027	0.021	0.25–0.49	0.35
	I	7	0.017–0.025	0.022	0.19–0.45	0.32
Corn						
white, whole kernel	C	40	0.010–0.039	0.022	0.46–1.50	0.88
	I	6	0.010–0.023	0.017	0.86–1.04	0.90
Yellow, whole kernel	C	47	0.015–0.045	0.027	0.56–0.95	0.75
	I	14	0.014–0.037	0.021	0.53–1.06	0.81
Peas	C	80	0.056–0.188	0.119	0.62–2.69	1.10
	I	20	0.078–0.146	0.103	0.42–1.34	0.96
Spinach	C	20	0.010–0.041	0.021	0.20–0.64	0.32
	I	11	0.009–0.025	0.018	0.16–0.51	0.27
Tomatoes	C	58	0.019–0.061	0.047	0.41–0.97	0.68
	I	5	0.045–0.077	0.063	0.74–0.96	0.86

[1] C = consumer size can.
[2] I = institutional size can.

The thiamine content did not seem to be correlated with the time of season except in the case of sweet peas. Early season peas were found to be slightly lower in thiamine content than mid-season and late-season samples. Average values from seventeen factories were 14.1% higher at mid-season and 11.5% higher at late season than the early season samples. It should be noted that because of the relatively high thiamine content of peas such differences are more readily detected than in the other products. The pH of the liquid in the can did not seem to be a factor in the variations found in the thiamine content of any one

product. It would seem that the longer sterilization times used for the larger institutional can sizes might reduce the thiamine content to lower values than those found in consumer size cans. The comparison in table 2, however, fails to show a consistent difference.

As expected, the fish products were found to contain more niacin than any other type of food analyzed. The values found ranged from 10.2 mg. % in tuna fish to 1.36 mg. % in shrimp. Of the canned vegetables analyzed, asparagus, corn, peas, and tomato juice were found to contribute the most niacin to the dietary, but were still relatively low in this vitamin since they contained only 0.75–1.06 mg. %. Fruits as a group were still lower, less than 0.7 mg. % being present.

As in the case of thiamine, there was no difference in niacin content of foods packed in the larger or smaller cans, as may be seen by comparing the average values in table 2. An attempt was made to correlate the niacin values found for each type of food with the time of harvest. In no case was any correlation found which would help to explain the wide ranges of some of the products. These ranges, as in the case of the thiamine assays, varied from 2- to 6-fold.

Although in the case of most foods the range is rather wide, this wide range is due to only a few samples. Thus the niacin content of peas in over 88% of the samples analyzed fell within a 2-fold range, although the extreme range was about 6-fold.

SUMMARY

Average values and ranges for the thiamine and niacin content are reported for thirty-two canned food products. Considerable variation has been found. The thiamine content of peas appears to be slightly affected by the packing season. With this exception no correlation was found between thiamine or niacin content and can size, time of harvest, or pH of these products.

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THE NUTRITIVE VALUE OF CANNED FOODS

IV. RIBOFLAVIN AND PANTOTHENIC ACID [1]

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A more thorough knowledge of the vitamin content of canned foods is of obvious importance to those who formulate nutritional programs and dietaries. In an effort to obtain reliable data, the National Canners Association — Can Manufacturers Institute, have collected and distributed samples of canned foods to a number of laboratories for assay. The method of collection, distribution, etc., has been described in detail in a previous report of this series (Clifcorn, '44). This laboratory has concerned itself with analyses of these samples for riboflavin and for pantothenic acid.

EXPERIMENTAL

Preparation of samples

Detailed treatment of samples varied somewhat with the nature of the material assayed. In all cases, contents of all cans of a particular sample (usually six) were mixed. Ten grams of the mixed samples of juice (grapefruit, orange, pineapple, tomato) were then taken directly for enzyme treatment. With apricots, asparagus, beets, grapefruit segments, peaches, pears, pineapple, spinach and tomatoes, the entire sample was mixed in an earthenware jar with a propeller mixer until the state of subdivision was rather fine (about 5 minutes). Four hundred and fifty grams of this material were then transferred to a Waring Blendor, and mixed for 3 to 5 minutes longer until the material was thoroughly homogenized. A 10-gm. sample of this material was then removed for enzyme treatment. Baked beans, salmon, sardines in tomato sauce, and shrimp (dry-pack) were hand-mixed; 225 gm. were then transferred to the Blendor, 225 gm. of water added, the mixture blended as above, and 10 gm. of the blended material (equivalent to 5 gm. of the original sample) removed for enzyme treatment. With mack-

[1] This work was supported in part by a grant from the National Canners Association — Can Manufacturers Institute Nutrition Program to Professor R. J. Williams (see Clifcorn, '44).

erel, sardines in oil, shrimp (wet-pack) and tuna, the liquid content of each can was discarded and the samples then mixed and treated in the same manner as the fish products above. With green beans, lima beans, carrots (diced), white and yellow corn, and peas, the liquid was drained off, and liquid and solid weights obtained. The solids were hand-mixed, a 450-gm. sample was reconstituted from the liquids and solids, and transferred to the Blendor, homogenized 3–5 minutes, and then a 10-gm. sample removed for enzyme treatment. Prunes were treated in the same manner as corn and peas, after preliminary removal of the pits.

For enzyme treatment, solutions of clarase and papain (Caroid) were prepared which contained 20 mg. of the enzyme dissolved (or uniformly suspended) in 1 ml. of acetate buffer, pH 4.5 (Cheldelin et al., '42). Five milliliters of each enzyme solution (equivalent to 100 mg. of each enzyme) were added to each 10-gm. sample of foodstuff. The mixture was diluted to 30 ml. with acetate buffer, and incubated for 24 hours at 37°C. under about 2 ml. of benzene. After incubation, the samples were heated at 100°C. for 15 minutes in flowing steam in order to remove benzene and precipitate proteins and then were diluted to 50 ml. with water. A small amount of Hyflo Supercel was added, and the samples then filtered with gentle suction. Assays for riboflavin and pantothenic acid were made on the filtrates. "Blank determinations" of the riboflavin and pantothenic acid concentrations in the enzyme samples were made by the same procedure at frequent intervals; this correction was subtracted from the values obtained on the sample. This correction varied slightly with different lots of enzyme, but averaged .032 mg. calcium pantothenate and .012 mg. riboflavin per 100 gm. of sample.

Assay methods

Riboflavin was determined by the method of Snell and Strong ('39); pantothenic acid was determined by the method of Pennington, Snell and Williams ('40). All assays were carried out on clear, filtered, enzymatic digests of the food sample, prepared as described above. Filtration was carried out at pH 4.5–4.7, and thus the presence of fatty materials which interfere in assays involving use of L. casei (Strong and Carpenter, '42; Wegner et al., '42) was avoided.

In general, the samples were satisfactory for the riboflavin assay. With the pantothenic acid assay, there was in many instances a drift in the values obtained. When this was present, those values calculated from low assay levels were considerably higher than those calculated

from higher levels. The values obtained tended to become constant at the higher assay levels (lowest assay figures). It was first attempted to remedy this situation by applying to the samples the method of Strong, Feeney and Earle ('41) and that of Landy and Dicken ('42). However, the same tendency was present to about the same degree when these methods were used. For purposes of assay the drift was therefore allowed to occur, but when it occurred only the lower values were used. These were obtained on the upper portion of the standard curve, where the drift in values was largely eliminated. Recovery experiments with added pantothenic acid showed 100% recovery at these levels, indicating validity of the results. Previous experience with microbiological assay methods has indicated that when such a drift occurs, the lower values, obtained at higher assay levels, approach more closely the true vitamin content of the material in question. Toward the end of the assay program, a modified method for pantothenic acid appeared [2] (Neal and Strong, '43). This method was sensitive to smaller amounts of pantothenic acid, and also largely eliminated drift in samples where this was apparent. Numerous assays were therefore made according to this procedure. Excellent agreement between results obtained by this method and the method used throughout the present investigation indicates reliability of results obtained with either technic (cf. table 2).

RESULTS AND DISCUSSION

A summary of the assay results obtained is given in table 1. Comparative assay results obtained by the method of Neal and Strong ('43) and that of Pennington, Snell and Williams ('40) are given in table 2.

One of the most interesting facts to emerge from these assays is the great variation in vitamin content which occurs from one sample to another of any one foodstuff. Examination of table 1 reveals that different samples of most products vary 3- to 4-fold in their vitamin contents; in some cases even greater variation is evident. This variation becomes more significant when it is remembered that most of the samples assayed consisted of the pooled contents of six cans of material. It emphasizes the unsatisfactory nature of conclusions based upon assays of the contents of individual cans, picked at random from shelves of retailers. The range as given in the table, however, is somewhat misleading, since it represents the extreme variations which occurred. Assays on the great majority of samples lay much closer to the average than these extremes would indicate.

[2] The authors wish to thank Dr. Strong for use of his manuscript in advance of publication.

Information supplied with the samples permitted their classification according to can size and packing date — early, late, or mid-season. Where the number of samples justified it, classification was made on each of the above bases. No significant differences in average values obtained appeared in any case.

TABLE 1

Pantothenic acid and riboflavin content of canned foods.

PRODUCT	NO. OF SAMPLES		CALCIUM PANTOTHENATE		RIBOFLAVIN	
	Retail size cans	cans No. 10	Range	Average	Range	Average
			mg./100 gm.	*mg./100 gm.*	*mg./100 gm.*	*mg./100 gm.*
Apricots, unpeeled halves	21		.045–.20	.095	.012–.039	.024
Asparagus, all green	20	11	.072–.43	.19	.039–.19	.096
Culturally bleached	8	4	.092–.16	.12	.044–.073	.058
Beans, baked						
New England style		3	.061–.11	.081	.051–.059	.054
With tomato sauce		2	.092–.11	.10	.019–.029	.024
Green cut	53	19	.023–.11	.061	.018–.065	.034
Lima, green	17	9	.068–.17	.11	.023–.062	.042
Beets	18	9	.045–.12	.076	.011–.059	.025
Carrots	12	7	.093–.21	.13	.009–.042	.021
Corn, white, whole kernel	40	6	.10 –.50	.18	.027–.064	.043
Yellow, whole kernel	47	14	.094–.32	.21	.025–.072	.044
Grapefruit juice	43		.065–.17	.12	.010–.033	.019
Segments	24		.063–.19	.13	.008–.039	.021
Mackerel	9		.13 –.48	.29	.13 –.29	.20
Orange juice	15		.081–.17	.12	.012–.038	.022
Peaches, clingstone, halves	17		.017–.065	.041	.013–.030	.022
Freestone, halves	14		.020–.13	.052	.009–.034	.021
Pears, halves	30		.008–.041	.022	.009–.032	.019
Peas, sweet, wrinkled varieties	73	21	.081–.26	.15	.025–.10	.054
Alaska	6	1	.069–.18	.13	.037–.064	.049
Pineapple juice	18		.066–.18	.10	.004–.031	.018
Sliced	17		.053–.15	.10	.006–.030	.021
Prunes, Italian [1]	10		.027–.085	.044	.019–.032	.026
Salmon	5		.47 –.73	.57	.14 –.17	.16
Sardines, in oil	5		.44 –.65	.53	.09 –.15	.11
In tomato sauce	10		.41 –.58	.47	.12 –.23	.18
Shrimp, dry pack	3		.26 –.35	.29	.027–.037	.032
Wet pack	5		.18 –.22	.21	.026–.035	.031
Spinach	20	11	.021–.14	.051	.024–.13	.082
Tomatoes	58	5	.11 –.44	.23	.011–.050	.028
Tomato juice	79		.17 –.39	.25	.009–.046	.028
Tuna	6		.13 –.19	.17	.11 –.17	.14

[1] Reported on pit-free basis. The pitted samples weighed on the average 95% as much as the whole sample.

It is well known that pantothenic acid occurs naturally in forms which are unavailable for test organisms used in microbiological assays. For this reason, enzymatic digestion of samples is now universally employed to liberate additional pantothenic acid. The enzymatic digestion procedure used throughout this work was that of Cheldelin et al. ('42). Application of this method to animal products results in large increases in pantothenic acid over those obtained by water extraction. At the time this assay program was undertaken, no investigation of the efficiency of enzymatic digestion procedures in liberating

TABLE 2

Comparative assays for pantothenic acid.

PRODUCT	NO. OF SAMPLES	CALCIUM PANTOTHENATE			
		Pennington et al.		Neal and Strong	
		Range	Average	Range	Average
		mg./100 gm.	*mg./100 gm.*	*mg./100 gm.*	*mg./100 gm.*
Beans, green cut	6	.060–.075	.065	.059–.090	.066
Green lima	3	.11 –.13	.12	.06 –.14	.10
Beets	1		.071		.070
Carrots	1		.13		.14
Corn, white, whole kernel	7	.16 –.50	.26	.16 –.42	.27
Yellow, whole kernel	13	.20 –.32	.23	.19 –.30	.26
Pears	1		.017		.019
Peas, sweet, wrinkled varieties	4	.13 –.15	.14	.06 –.20	.14
Pineapple, sliced	1		.08		.10
Sardines, in oil	1		.49		.56
Shrimp	4	.20 –.26	.22	.20 –.29	.22
Spinach	1		.039		.083
Tomatoes	1		.23		.30
Tomato juice	2		.27		.27
Tuna	2		.19		.19

pantothenic acid from cooked plant or animal tissues had been made. It is possible that other digestion procedures would liberate more pantothenic acid than that indicated by our assays. The figures obtained here for pantothenic acid should thus be regarded as minimum values for the canned product in question.

The use of enzymatic digestion preliminary to riboflavin assay is not so common. For the above program, use of such a procedure presented the very considerable advantage that the preparation of only one extract instead of two was necessary. Preliminary trials with a number of samples showed that values obtained by the above procedure checked those obtained by the more commonly used procedures of autoclaving

with water (Snell and Strong, '39) or with dilute acid (e. g. Wegner et al., '42). Cheldelin et al. ('42) also found enzymatic digestion to be as efficient as neutral- or acid-autoclaving in liberating riboflavin, while digestion with pepsin was recommended by Van Duyne ('41) for the extraction of riboflavin from tissues.

With both pantothenic acid and riboflavin, the correction in assay value rendered necessary by the vitamin content of the enzyme preparation used is considerable; in some cases it approaches in magnitude the vitamin content of the product in question. Values for this correction are entirely reproducible, however, so that the procedure would lead to error only if the value for the enzyme correction considerably surpassed the assay value of the sample. This situation did not exist. That this factor did not influence the accuracy of the assays is shown by the agreement of values obtained by two methods for pantothenic acid (table 2), and the above-mentioned agreement in assay values for riboflavin obtained after enzyme-treatment, and after extraction with acid.

SUMMARY

Average values for the riboflavin and pantothenic acid content of 32 different types of canned foods are given. The amount of these vitamins present in different samples of any given canned food varies considerably. Reasons for this variation are not apparent from the present study. It was not correlated with can-size, or with the time during the growing season that the food was packed.

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THE NUTRITIVE VALUE OF CANNED FOODS

V. DISTRIBUTION OF WATER SOLUBLE VITAMINS BETWEEN SOLID AND LIQUID PORTIONS OF CANNED VEGETABLES AND FRUITS

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This portion of the research program of the National Canners Association — Can Manufacturers Institute was designed to supplement the large body of data on vitamin contents of a great variety of canned foods to be obtained by other laboratories, with information about the distribution of water-soluble vitamins in a representative number of canned vegetables and fruits. A fair picture of such distribution could be obtained, it was thought, by determination of three of these vitamins; namely, ascorbic acid, thiamine, and riboflavin in the solid and liquid portions of several types of the canned products. Thus the proportions of the three vitamins found in the solid and in the brine of eight different canned vegetables, both in consumer size and in no. 10 size cans, as well as those found in the solid and in the syrup of seven canned fruits, in consumer size cans only, are here reported.

EXPERIMENTAL METHODS

Separation and weighing of solid and liquid

Separation of solid and liquid portions for distribution studies was carried out approximately according to the standard techniques described by the Association of Official Agricultural Chemists. The only variations from these procedures were that a 12-inch sieve, rather than an 8-inch one, was used for drainage of whole single no. 2 or no. 2½ cans, and that for all vegetables in no. 10 cans, except spinach, the contents of each can were drained in two portions, each for 2 minutes, on the 12-inch sieve. For no. 10 cans of spinach the contents of a can were drained in three portions, each for 3 minutes.

The weights of solid and liquid recorded in the tables on the "per can" basis are, in the case of consumer sizes, the single can averages of the weights obtained on mixtures of solids and mixtures of liquids combined from three or six cans, and are, in the case of no. 10 cans, the averages of six separate weighings of the two portions of six no. 10 cans.

Sampling for analyses

For vegetables in consumer size cans, the whole amount of solids from three (or six) cans was mixed as well as possible without macerating the material and then two approximately 150- to 200-gm. samples were removed from the mixture and separately blended in the Waring Blendor with measured amounts of 3% metaphosphoric acid and 0.1 N sulfuric acid in preparation for the extraction of ascorbic acid and thiamine-riboflavin respectively. The whole lot of solid from three such cans was deliberately not more intimately mixed, as by maceration, before the samples were withdrawn, because, for the other part of this study, the remaining two can quantities of solid were required intact. These were to be further divided for reconstituting with liquid to form representative single can contents which would be used in duplicate experiments on vitamin retentions in heating as in family scale preparation for serving.

For vegetables in no. 10 cans, 200-gm. samples were removed from each can's solid portion before the remainders were combined for the experiments on large scale preparation for serving. The 1200 gm. thus brought together from six cans were thoroughly mixed with a beater in the "Kitchen Aid" bowl and the 200-gm. samples then used for vitamin determinations were taken into the Waring Blendor cup from this macerated mixture.

For fruits, no heating experiments were carried out and since in these the solid portions were all large pieces, the mixture of solids from six consumer size cans were necessarily also macerated in the "Kitchen Aid" bowl before samples were taken for analyses.

Sampling of solid portions was further continued by transferring appropriate aliquots of blends, to volumetric flasks for buffering in the case of ascorbic acid determination, and to 250-ml. centrifuge bottles for hot extraction with 0.1 N sulfuric acid in the case of thiamine and riboflavin determinations.

In sampling the liquid, the weighed portions were withdrawn from the complete mixture of liquid from all the cans of a set, and were transferred to the appropriate acids. For ascorbic acid extracts, 25- to 50-gm. samples were taken and for thiamine-riboflavin digests, 15- to 30-gm. samples.

Methods of analyses

Chemical methods of analyses were employed for determination of the three vitamins.

For most of the ascorbic acid determinations, the Morell ('41) modification of the Bessey ('38) method for photoelectric measurement of the reductions of the indophenol dye was used, employing the Coleman Universal Spectrophotometer Model II. For these, the wave length dial was set at 500 mμ, since that was found to be the wave length of maximum absorption for the particular batch of dye used throughout the experiments. The calibration equation derived by the method of least squares was used for calculations, rather than a plotted curve. However, even with the use of such an equation, dilutions of ascorbic acid in the final test solution below 0.002 mg, per milliliter were avoided, since determinations below this level were apt to have errors in excess of 10%.

In the first four experiments, namely, those on sweet peas in table 1, the Bessey ('38) technique was used, with the wave length dial set at 520 mμ. In these experiments, to avoid unduly large errors, the concentration of ascorbic acid in the final test solution was not allowed to fall below 0.004 mg. per milliliter.

Finally, because the Spectrophotometer had to be sent away for a galvanometer repair, it was necessary to use the titration method in a considerable number of determinations averaged in table 1; namely, those on green beans, all of those on corn, and in one on spinach. In these, a 3% metaphosphoric acid extract was titrated to a 15-second end-point with the dye.

For thiamine and riboflavin determinations, acid digestion was followed by overnight incubation with 0.4% clarase at 37°C. and ph 4.3 to 4.5. Then the double adsorption technique of Conner and Straub ('41) was used.

Since we have had some difficulty regarding satisfactory recovery of the vitamins from recent samples of their respective adsorbents and have learned that others have likewise had trouble, it would seem to be appropriate to state here that all samples of Decalso and Florisil employed in this study had been tested with solutions of crystalline thiamine and riboflavin respectively, and that no adsorbent was put into use which gave less than 93% recovery. Moreover, with each set of determinations on a vegetable or fruit, one recovery experiment was included, in which synthetic thiamine and riboflavin were added to a digestion bottle along with a third aliquot of the blend of the solid sample and carried through the whole procedure. Among forty-eight such determinations, thiamine recoveries ranged from 90 to 107% in forty-one of them and were 75, 75, 84, 109, 113, and 116% in the remaining seven, whereas riboflavin recoveries ranged from 85 to 106%

TABLE 1

Distribution of water soluble vitamins in consumer size cans of vegetables.[1]

VEGETABLE	NO. OF EXPERIMENTS IN AVERAGES		WEIGHT		ASCORBIC ACID		THIAMINE		RIBOFLAVIN	
			Total per can	Distribution	Concentration	Distribution	Concentration	Distribution	Concentration	Distribution
			gm.	%	*mg./100 gm.*	%	*mg./100 gm.*	%	*mg./100 gm.*	%
Asparagus, all green	1	Solid	359	62	17.6	60	0.065	62	0.124	71
		Liquid	222	38	19.2	40	0.065	38	0.081	29
Beans, green cut	5[2]	Solid	375	64	5.41	64	0.036	67	0.054	76
		Liquid	212	36	5.54	36	0.032	33	0.030	24
Beans, lima, green	4[3]	Solid	411	70	5.91	56	0.028	68	0.054	76
		Liquid	178	30	10.3	44	0.030	32	0.039	24
Carrots	1	Solid	378	64	2.55	66	0.022	66	0.021	74
		Liquid	216	36	1.97	34	0.020	34	0.013	26
Corn, white whole kernel	1	Solid	392	67	3.86	48	0.015	67	0.031	80
		Liquid	191	33	8.54	52	0.015	33	0.026	20
Corn, yellow whole kernel	4[4]	Solid	405	68	5.19	61	0.034	67	0.063	78
		Liquid	186	32	7.18	39	0.035	33	0.036	22
Peas, sweet wrinkled varieties	4[5]	Solid	393	66	9.30	63	0.120	66	0.065	70
		Liquid	203	34	10.6	37	0.121	34	0.054	30
Spinach	2[6]	Solid	495	63	5.93	62	0.013	69	0.080	76
		Liquid	296	37	6.15	38	0.014	31	0.042	24

[1] For asparagus, carrots, and white corn, six no. 2 cans were mixed; for each experiment with green beans, lima beans, yellow corn, and sweet peas, three no. 2 cans were mixed; and for each experiment with spinach, six no. 2½ cans.

[2] Except for ascorbic acid, 4 experiments.

[3] Except for ascorbic acid, 2 experiments.

[4] Except for thiamine, 3 experiments, and for riboflavin, 2 experiments.

[5] Except for thiamine, 3 experiments.

[6] Except for thiamine, 1 experiment.

[2] Except for thiamine, 1 experiment.

TABLE 2

Distribution of water soluble vitamins in no. 10 cans of vegetables.[1]

VEGETABLE		WEIGHT		ASCORBIC ACID		THIAMINE		RIBOFLAVIN	
		Total per can	Distribution	Concentration	Distribution	Concentration	Distribution	Concentration	Distribution
		gm.	%	*mg./100 gm.*	%	*mg./100 gm.*	%	*mg./100 gm.*	%
Asparagus, all green	Solid	1888	62	13.1	63	0.077	62	0.114	69
	Liquid	1147	38	12.8	37	0.077	38	0.086	31
Beans, green cut	Solid	1848	61	2.35	61	0.028	67	0.040	74
	Liquid	1183	39	2.37	39	0.022	33	0.023	26
Beans, lima, green	Solid	2309	71	7.02	58	0.020	67	0.055	76
	Liquid	942	29	12.5	42	0.025	33	0.044	24
Carrots	Solid	2203	73	1.28	68	0.022	72	0.024	78
	Liquid	846	27	1.61	32	0.022	28	0.018	22
Corn, white whole kernel	Solid	2013	64	4.70	54	0.010	61	0.051	76
	Liquid	1148	36	6.93	46	0.011	39	0.029	24
Corn, yellow whole kernel	Solid	1985	64	2.09	46	0.012	63	0.062	72
	Liquid	1103	36	4.36	54	0.012	37	0.044	28
Peas, sweet wrinkled varieties	Solid	2083	69	7.43	62	0.112	68	0.077	73
	Liquid	970	31	10.00	38	0.118	32	0.062	27
Spinach	Solid	1497	51	16.2	47	0.024	53	0.132	60
	Liquid	1428	49	19.0	53	0.022	47	0.094	40

[1] For all vegetables, six no. 10 cans were mixed in each experiment and each average value is calculated from two such experiments.

in forty out of forty-seven determinations and were 73, 82, 82, 110, 110, 111, and 116 in the remainder.

For fluorescence readings the Coleman Electronic Photofluorometer was used. For standardizing the instrument sensitivity the usual stable fluophor, quinine sulfate (0.135 μg. per milliliter) in 0.1 N H_2SO_4 was used for the thiochrome readings, whereas sodium fluorescein (0.100 μg. per milliliter) in 0.01 N NaOH was used for riboflavin.

The most important of the variations made by us from the rest of the Conner and Straub procedure concerned the fluorescence determinations in the eluates of thiamine and riboflavin. For both, individual non-specific fluorescence blank readings were made; in the former, by the usual Hennessy and Cerecedo ('39) technique of omitting the potassium ferricyanide; in the latter, by exposing some of each eluate to light (Najjar, 41), using a General Electric AH-4 100-watt mercury vapor lamp in an ordinary pyrex test tube at a distance of 12 mm. from the lamp bulb for a period (22 to 30 min.) which in preliminary tests, had been determined as sufficient to destroy all the riboflavin, following which the same $KMnO_4$ and H_2O_2 treatment used on an unexposed aliquot of eluate was also given to the blank. Further appropriate corrections were also made on all readings for thiamine and riboflavin fluorescence values of those amounts of clarase represented in the final test aliquots. Moreover, for both vitamin determinations internal standard readings were taken as the basis for calculation, using the average of the increments in readings in a series, obtained by the addition of 0.2 μg. (or 0.4) of thiamine and 1.0 μg. (or 0.5) of riboflavin to one aliquot from each of the respective eluates.

DISCUSSION OF RESULTS

Vegetables

Duplication of vitamin values in two lots from the same pack. Vitamin concentration values in duplicate 3-can lots of consumer size cans and in duplicate 6-can lots of no. 10 cans of identical code numbers were in general, in good agreement for thiamine and riboflavin, but not so good for ascorbic acid in nos. 2 and 2½ cans and still less satisfactory for ascorbic acid in no. 10 cans.

For thiamine and riboflavin, the variations between such duplicate lots for all can sizes were not over 10% except in five cases out of twenty-four for each vitamin, these running between 11 and 21% for the thiamine and between 13 and 33% for riboflavin.

For ascorbic acid the variation in duplicate lots of nos. 2 and 2½ cans was over 20% in only one sample out of fourteen, whereas, in

no. 10 cans there were six instances out of a possible ten in which variations were over 20% and reached as high as 40%.

Comparative concentrations of vitamins in solids and liquids (all can sizes). The concentration of ascorbic acid in the solid ran generally somewhat lower than in the liquid, the differences being most pronounced (50% to 100% of the solid value) for such products as corn and lima beans, in which a relatively lower water content of the solid portion probably explains the exaggerated difference in the amount of ascorbic acid dissolved in the two phases.

The concentrations of thiamine in solid and liquid were the same, within experimental error, with eight exceptions among thirty-five possibilities. In the eight exceptional determinations, the variations between thiamine values for solids and liquids ran from 20% to 33%, and in both directions.

The concentration of riboflavin in the solid was considerably higher than in the liquid in all cases, running higher from about 15% to 20% of solid value in the case of the solid of peas and some lima beans, to 40% to 50% in the case of the solid of some samples of green beans, corn and spinach.

Distribution of weight, ascorbic acid, thiamine and riboflavin between solid and liquid. For all sizes of cans and all vegetables except spinach, and one lot of asparagus in no. 10 cans, the solid weight was in the range of 60% to 73%. One 6-can lot of no. 2½ cans of spinach had solid weight of 55% and two 6-can lots of no. 10 cans of spinach had solid weight of 48% and 54%, whereas the exceptional lot of asparagus contained 58% of solid.

For all sizes of cans: in asparagus, green beans, carrots and peas, the solid carried 60% to 68% of the ascorbic acid; whereas, in white and yellow corn, lima beans, and spinach, the solid carried 46% to 58% of the ascorbic acid. However, in consumer size cans there was one lot out of two, of yellow corn and spinach which fell in the range of the first group of vegetables in their ascorbic acid distribution.

For all sizes of cans and all vegetables, except some asparagus and corn and all spinach in no. 10 cans, the solid carried from 62% to 72% of the thiamine. In one out of two 6-can lots each of asparagus and white corn in no. 10 cans, the solid carried 58% and 59%, respectively, whereas for spinach in no. 10 cans the average was 53%.

For all sizes of cans, and all vegetables except spinach, the solid carried 70% to 80% of the riboflavin. Spinach varied a great deal, for in no. 2½ cans the solid had 69% and 82% of the riboflavin, whereas in no. 10 cans it varied from 57% to 62%. In one out of two lots of

TABLE 3

Distribution of water soluble vitamins in consumer size cans of fruits.[1]

FRUIT		WEIGHT		ASCORBIC ACID		THIAMINE		RIBOFLAVIN	
		Total per can	Distribution	Concentration	Distribution	Concentration	Distribution	Concentration	Distribution
		gm.	%	*mg./100 gm.*	%	*mg./100 gm.*	%	*mg./100 gm.*	%
Apricots, unpeeled halves (ripe)	Solid	467	53	3.25	53	0.020	50	0.012	65
	Liquid	419	47	3.21	47	0.022	50	0.011	45
Grapefruit segments	Solid	347	57	23.0	56	0.034	58	0.006	67
	Liquid	262	43	24.0	44	0.032	42	0.004	33
Peaches, clingstone	Solid	567	67	4.81	67	0.007	70	0.012	61
	Liquid	284	33	4.68	33	0.006	30	0.015	39
Peaches, freestone	Solid	431	51	2.42	49	0.006	61	0.011	56
	Liquid	417	49	2.64	51	0.004	39	0.009	44
Pears	Solid	518	61	1.18	68	0.006	57	0.011	69
	Liquid	329	39	0.86	32	0.007	43	0.008	31
Pineapple, sliced	Solid	523	60	4.76	59	0.077	60	0.004	66
	Liquid	352	40	4.89	41	0.078	40	0.003	34
Prunes, italian	Solid	383	46	0.96	49	0.022	51	0.012	53
	Liquid	456	54	0.85	51	0.018	49	0.009	47

[1] Six no. 2½ cans were mixed for all fruits except grapefruit for which six no. 2 cans were used. Each value is from a single experiment.

asparagus, in no. 10 cans, the solid also had less of the riboflavin, 64%.

Fruits

Comparative concentrations of vitamins in solids and liquids. (Consumer size cans only). In contrast to vegetables, the ascorbic acid concentration in the solids of canned fruits was the same, within experimental error, as in the liquids except in the case of pears. For these the solid had a 20% higher value than the liquid.

As for vegetables, thiamine concentrations are about the same in solids and liquids, except possibly in prunes, in which the 20% higher value for solids should be significant, the difference being in excess of experimental errors.

Also as for vegetables, riboflavin values for solids are higher than those for liquids in all fruits except cling peaches. Although in most instances the percentage differences are as great as for vegetables, they are not as significant, especially for the lowest values, because of the large experimental errors in such low values.

Distribution of weight, ascorbic acid, thiamine and riboflavin between solid and liquid. The whole range of solid weight is 46% to 67%, with prunes and freestone peaches lowest and cling peaches highest. There are too few values to break them down into groups.

The total ascorbic acid carried by the solid follows quite closely the percentages of solid weights and therefore the values are also difficult to group. In pears, however, higher ascorbic acid concentration in the solid which has been noted, shows up in the percentage figure for ascorbic acid in the solid.

The total thiamine carried by the solid also follows the percentages of solid weight, being significantly higher only for prunes, as might be expected from the concentration values, and for freestone peaches, where the difference may be due to unduly large errors in the extremely low thiamine values.

The total riboflavin carried by the solid is by percentage 5% to 12% higher than the percentage of solid weight, except, of course, in cling peaches, where it is lower in accordance with its lower concentration in solid.

SUMMARY

To obtain information about the distribution of water-soluble vitamins in canned vegetables and fruits, determinations of ascorbic acid, thiamine and riboflavin were made on the drained solids and liquids of mixed 3-can or mixed 6-can lots of identical pack, including eight dif-

ferent vegetables, both in consumer size and in no. 10 size cans, and seven different fruits in consumer size cans. The degree of agreement between the concentrations of these vitamins in duplicate lots of identical pack and the comparison of the concentrations in solids and liquids are summarized. Regarding the over-all distributions, the observations led to the following general conclusions: (1) in most canned vegetables the solid weight, being 60% to 73% of the total can contents, carried 46% to 68% of the ascorbic acid, 62% to 72% of the thiamine, and 70% to 80% of the riboflavin. Spinach was the outstanding exception to these ranges, for with solid weights ranging from 48% to 55% of the total, all vitamin contents of the solid were correspondingly lower. (2) In fruits, the solid weights of the packs showed more variation, being 46% to 67%, and the vitamin percentages borne by these solids were consequently also more variable, ascorbic acid and thiamine percentages in solid agreeing closely with the weight percentages, and riboflavin paralleling them at about 5% to 12% higher level.

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